Evaluation of clinical functional magnetic resonance imaging (fMRI) applications within the mesial temporal lobe

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Abbreviations

AED	Antiepileptic drug
ANOVA	Analysis of variance
BOLD	Blood-oxygen-level-dependent
CBF	Cerebral blood flow
EEG	Electroencephalography
EPI	Echo planar imaging
fMRI	Functional magnetic resonance imaging
GLM	General linear model
HS	Hippocampal sclerosis
IAT	Intracarotid amobarbital test
ICC	Intraclass correlation coefficient
LI	Lateralisation index
M1	Primary motor area
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTL	Mesial temporal lobe
MTLE	Mesial temporal lobe epilepsy
PET	Positron emission tomography
RMANOVA	Repeated measurement analysis of variance
ROI	Region of interest
SNR	Signal-to-noise ratio
SPECT	Single photon emission tomography
T	Tesla
V1	Primary visual area
VOI	Volume of interest

1 Zusammenfassung

Das Ziel der vorliegenden Arbeit war, klinische Anwendungen der funktionellen Magnetresonanztomographie (fMRT) im mesialen Temporallappen (MTL) zu untersuchen, um damit die klinische Diagnostik bei der mesialen Temporallappenepilepsie (MTLE) zu verbessern. Die MTLE ist die häufigste Form fokaler Epilepsien (Engel et al 1997). Deren Behandlung erfolgt mit Hilfe antikonvulsiver Medikamente (AED) oder, wenn die Anfälle trotz medikamentöser Behandlung weiterbestehen, mit einem epilepsiechirurgischen Eingriff. Beide Behandlungsmethoden können die kognitiven Funktionen eines Patienten beeinträchtigen. Weil intakte kognitive Funktionen wichtig für die Lebensqualität sind (Trimble 1994), ist eine sorgfältige und umfassende Untersuchung von möglichen Therapieeffekten unabdingbar. Dies wird durch die neuropsychologische Diagnostik, die sich zunehmend auch fMRT zu Nutze macht, bewerkstelligt.

Individualdiagnostische fMRT-Untersuchungen sind wegen der schwachen Signalstärken, insbesondere im Bereich der mesialen Temporallappen (MTL), problematisch. Eine mögliche Lösung dieses Problems könnte sein, das Signal pharmakologisch zu verstärken. Weil diesbezüglich positive Erfahrungen mit Koffein in primär motorischen und visuellen Arealen vorliegen (Mulderink et al 2002), wurde in einer ersten Studie untersucht, ob Koffein geeignet ist, in der klinischen fMRT-Individualdiagnostik das Signal zu verstärken. Mit Hilfe einer Längsschnittstudie (pro Versuchsperson acht Wiederholungsmessungen mit vier Baseline-, zwei Placebo- und zwei Koffeinmessungen) wurden drei funktionelle Domänen, das primär visuelle und das primär motorische Areal sowie die MTL untersucht. In unserer Studie war Koffein viel weniger wirksam als erwartet, insbesondere konnten wir im MTL keinen Einfluss nachweisen. Vielmehr wirkte Koffein domänenspezifisch, sogar innerhalb ein und derselben Versuchsperson und verstärkte zudem die interindividuelle Variabilität. Unsere Studie legt deshalb nahe, dass Koffein nicht als Signalverstärker für klinische fMRT-Studien verwendet werden sollte.

Hinsichtlich der medikamentösen Epilepsiebehandlung könnte der Gebrauch von fMRT die Diagnostik kognitiver Nebenwirkungen wesentlich verbessern, weil im Gegensatz zur neuropsychologischen Untersuchung mehrere konfundierende Variablen kontrolliert werden können (z.B. Anfälle, interiktale Entladungen, Stimmung). Bevor fMRT dafür eingesetzt werden kann, muss die Methode hinsichtlich ihrer längerfristigen Wiederholbarkeit validiert werden. Dieses Ziel wurde in der zweiten Studie verfolgt, indem die fMRT-Untersuchungen der Roland Hometown Walking Task (Jokeit et al 2001c) zur Aktivierung des MTL sechsmal wiederholt wurde. Es konnte gezeigt werden, dass es mit Hilfe serieller fMRT-Langzeitgruppenstudien möglich zu sein scheint AED Nebenwirkungen

nachzuweisen. Die Kenntnis der Variabilitätshöhe von wiederholten fMRT-Untersuchungen hilft in zukünftigen Studien zwischen der Variabilität, die durch die Methode erklärt werden kann und dem tatsächlichen Einfluss von Medikamenten zu unterscheiden.

Die dritte Studie befasste sich mit der funktionellen Darstellbarkeit der Amygdala im Rahmen der prächirurgischen fMRT-Diagnostik. Letztere wurde bisher vorwiegend dazu verwendet, Sprach- und Gedächtnisfunktionen zu orten und zu lateralisieren, um unerwünschte Folgen von Epilepsieoperationen zu vermeiden (Detre 2004). Es konnte gezeigt werden, dass es möglich ist, die Amygdala im Einzelfall funktionell zu untersuchen. Die dazu verwendete fMRT-Aufgabe beinhaltete Videosequenzen, in denen angsterfüllte Gesichter (Aktivierungsbedingung) und Landschaftsaufnahmen (Kontrollbedingung) zu sehen waren. Diese Aufgabe aktivierte die Amygdala individuell stark, zuverlässig, wiederholbar und spezifisch. Die individuelle Darstellbarkeit der Amygdala erlaubt zukünftig, klinische Konsequenzen der Resektion zu untersuchen. Dazu gehören zum Beispiel die emotionale und soziale Kognition, die eng mit der Funktionstüchtigkeit der Amygdala in Zusammenhang gebracht wird. Die Kombination der Amygdala-Messung mit einer Aufgabe zur Gedächtnismessung erhöht zudem die Zuverlässigkeit und Genauigkeit der Lateralisierung der epileptogenen Seite bei Patienten mit MTLE.

2 Summary

The present thesis was aimed at investigating clinical functional magnetic resonance imaging (fMRI) applications within the mesial temporal lobe (MTL). These applications were considered to improve the clinical fMRI diagnostics in mesial temporal lobe epilepsy (MTLE), the most frequent form of focal epilepsy (Engel 1996). Mesial temporal lobe epilepsy is mainly treated with antiepileptic drugs (AED) or, when the seizures are refractory, by resective surgery. Both therapies may have substantial influence on patients' cognitive profiles. A comprehensive MTLE care includes the evaluation and the prevention of negative treatment effects. This is provided by neuropsychological examination and increasingly by fMRI. The importance of evaluating the cognitive side-effects of treatments is based on the fact that disturbances of cognitive function are one of the major influences on quality of life in individuals with epilepsy (Trimble 1994).

One of the major difficulties in the application of fMRI in a clinical context is the low BOLD contrast in individual subjects, especially within MTL, where the SNR is genuinely low. Because caffeine has recently been proposed as an effective BOLD contrast booster in functional MRI studies for studies confronted with a low SNR (Mulderink et al 2002), we evaluated caffeine as a contrast booster for single-case fMRI-investigations. We chose an extended study design with each person acting as their own control in four baseline, two placebo and two caffeine measurements and investigated the effect of caffeine in primary visual, primary motor area and the MTL. We found that caffeine is a far less effective BOLD contrast booster than expected and we clearly failed to find any effects following caffeine administration within the MTL. Caffeine related BOLD-contrast changes were even more domain specific, even within the same subject. Moreover, caffeine may enhance inter-individual variability and reduce the signal in certain subjects. Our results therefore discourage the use of caffeine as a BOLD contrast booster in a clinical context.

Regarding the diagnostics of pharmacologically induced cognitive dysfunctions, we were able to plan future pharmaco-fMRI studies targeting cognitive side-effects of AEDs. The application of fMRI within this field could improve the diagnostics of pharmacologically induced cognitive side-effects. In contrast to neuropsychological testing, it allows one to examine cognitive side-effects directly and specifically in the MTL. The present study, which investigated the Roland Hometown Walking task (Jokeit et al 2001c) within the MTL over six serial fMRI-scans provides data on the variability inherent in the long-term-stability of fMRI results within MTL. Our study demonstrated that the application of fMRI in studying the effects of AED treatments on brain functions is possible, but it may be restricted to its application in longitudinal group studies with multiple scans. Knowledge

about the magnitude of variability aids in future AED studies in distinguishing between variations inherent in the examination method and changes in the subject's brain induced by pharmacological treatment.

The third study was aimed at expanding presurgical fMRI-diagnostics with the functional investigation of an additional structure, namely the amygdala. Functional MRI is used to examine regional changes in brain function associated with seizures. To date, presurgical fMRI is mainly used to assess localisation and lateralisation of language and memory to prevent postsurgical impairments (Detre 2004). Our study demonstrated the feasibility of additionally imaging the amygdala in presurgical fMRI-diagnostics in individual subjects. By employing dynamic fearful faces contrasted by bland landscapes our paradigm activated both amygdalae strongly, robustly, replicably and specifically in individual subjects. The ease of administration and the low cognitive demand on patients increases the practicality of this tool for studying amygdala-functions and deficits in a clinical context. The feasibility of imaging the amygdala in individual cases allows for the evaluation of possible clinical implications for amygdala-related functions including emotional and social processing following temporal lobe surgery. The combination of the fearful face paradigm and the Roland's Hometown Walking Task increases the reliability of lateralisation in MTLE patients and, therefore, provides a more detailed and reliable presurgical mapping of MTL structures.

3 Theoretical background

There is a substantial desire to apply functional magnetic resonance imaging (fMRI) to the non-invasive investigation of brain function in both basic and clinical neuroscience. Generally, the use of fMRI in a clinical setting presents challenges beyond those encountered in basic neuroscience, where mostly group studies with healthy volunteers are used. Problems include for example the small signal-changes or artefacts induced by motion or susceptibility. The distinct advantages of fMRI on the other hand include its non-invasiveness, relatively high spatial and for many purposes sufficient temporal resolution, ease of imaging the underlying anatomy, and the high accessibility of MR scanners.

In the present report, the application of fMRI in epilepsy is investigated. The use of fMRI in this context has received considerable attention for a variety of reasons. First, epilepsy is in many instances a functional disorder that must not necessarily be accompanied by gross abnormalities on structural imaging. Accordingly, fMRI has been used to examine regional changes in brain function associated with seizures, as has previously been demonstrated with radionuclide imaging. Second, fMRI has been used to assess localisation of language, memory and other functions as part of the preoperative evaluation for epilepsy surgery of patients with medically refractory epilepsy (Detre 2004). The use of fMRI in this context is very attractive, because it allows for the validation of fMRI-results with other brain mapping techniques (e.g. the intracarotid amobarbital test or electrocorticographic mapping) or the postsurgical outcome. Third and finally, fMRI allows for the study of pharmacologically induced effects (Honey et al 2004; Leslie et al 2000) and could in future be an attractive tool to study cognitive side-effects of antiepileptic drugs (AED).

3.1 The mesial temporal lobe epilepsy (MTLE)

In the following chapters, background information about mesial temporal lobe epilepsy (MTLE) will be provided, in order to support the understanding of fMRI application in this syndrome.

3.1.1 Neurological characteristics of MTLE

Mesial temporal lobe epilepsy is the most frequent form of focal epilepsy in adults (Engel et al 1997) and epileptic seizures typically arise from the inner part of the mesial temporal lobe (MTL) (Mathern et al 2002). Seizures are associated with specific anatomic changes in the deep areas of the temporal lobe (TL), including tissue shrinkage, cell loss, and

reactive gliosis (Mathern et al 2002). Neuron cells are replaced by the glial cells, leading to atrophy and sclerosis of the mesial temporal areas (Kalviainen et al 2002; Mathern et al 2002). Today it has been concluded that MTL sclerosis is both a cause and an effect of seizures.

Risk factors for MTLE include febrile seizures (frequently prolonged ones), meningitis, encephalitis, or head trauma (Annegers et al 1996; Jokeit et al 2004). There is often a latency of 5 to 10 years between the brain insult and the onset of seizures (Berg et al 2003; French et al 1993).

Seizure semiology in patients with temporal lobe epilepsy includes simple partial seizures (auras), complex partial, or secondarily generalised tonic–clonic seizures. The simple partial seizure is an ictal event generated predominantly by MTL structures. Conscious awareness is preserved. It usually involves symptoms referable to this area such as a sensation of epigastric rising, emotional changes (most commonly fear), and occasionally olfactory or gustatory hallucinations. The classical mesial temporal simple partial seizures characteristically occur in isolation, reflecting the fact that there is little transfer across the human hippocampal commissure, so that seizures originating in one hippocampus remain unilateral for long periods of time and may never propagate to the contralateral side to produce impairment of consciousness (Devinsky 2004; Engel 1996).

Complex partial seizures impair consciousness and memory. When these seizures occur, they also have the characteristic features of mesial temporal involvement. There is typically a stare with arrest of motion, followed by altered responsiveness, oroalimentary and gestural automatism, and later reactive automatism's lasting typically one to two minutes (Devinsky 2004; Engel 1996).

Secondarily generalised seizures can occur with or without prior simple or complex partial seizures. These seizures occur only rarely, probably due to the fact that currently used AEDs are effective against the ictal evolution that leads to these events but not against the simple and complex partial seizures of MTL origin (Devinsky 2004; Engel 1996).

For individual patients, TL seizures are usually stereotypic in their symptoms and duration. Postictal confusion, tiredness, and amnesia are common. Postictal symptoms can help to lateralise the focus (e.g. impairments in verbal memory and naming after left-sided seizures and geographic disorientation after right-sided seizures). Some patients are unaware of the mental lapses, denying any impairment in level of consciousness (Devinsky 2004; Engel 1996).

3.1.2 Neuropsychological characteristics of MTLE

Mesial temporal lobe epilepsy has become the best neuropsychologically investigated type of epilepsy because of its high prevalence and its underlying structural lesion in a circumscribed brain structure.

Mesial temporal lobe structures, i.e. hippocampus, parahippocampus and amygdala, differentially contribute to memory processing. The amygdala refers additionally to mood and emotional processing, including social cognition.

Memory

The leading functional symptoms of the majority of patients with MTLE are deficits of episodic memory (Eichenbaum 1999; McGaugh 2000). The term episodic memory refers to the cognitive processes that enable the explicit recollection of unique events and the context in which they occurred. These include the transformation of an experience into an enduring memory trace (memory encoding) and the subsequent recollection of the event at a later time (memory retrieval). These processes mainly depend on a functionally intact hippocampus (Baddeley 2001). The exact role of the hippocampus is still a matter of controversy. It is suggested that elemental cognitive processes, including processing associations, organising an episode as sequence of events and 'relational networking', mediate these complex memory functions. Within associative representations, the hippocampus seems to support both encoding (Sperling et al 2003) and retrieval processes (Maguire et al 2003) such as in the retrieval of autobiographical experiences. Even more, the level of detail, personal significance and emotionality seems to contribute to hippocampal activation of autobiographical memories (Addis et al 2004). 'Relational networking' terms the linking of episodic memories into relational networks in order to abstract the common features among related memories. Within this model, the hippocampus constructs relational networks that co-ordinate memories stored in the cerebral cortex (Eichenbaum 2004).

The role of the parahippocampus is suggested to held neocortical perceptually and conceptually processed information (Eichenbaum 2000) and is responsive for spatial memory such as visual imagery and retrieval of landmarks and their location (Rosenbaum et al 2004). Interestingly, the hippocampus is suggested not to be needed for the storage and recovery of a spatial layout. It seems to be engaged only in the retrieval of recent spatial memory, where the hippocampus binds details and therefore facilitates the extraction of schematic spatial information (Mellet et al 2000). In contrast, the

hippocampus does not participate in the retrieval of remote spatial memory, because the schematic representation is already completed and cortically represented (Rosenbaum et al 2004).

The amygdala finally is important in enhancing the retention of emotionally-valenced information (Zald 2003). This is best seen in the fact that emotionally arousing information is recalled and recognised better than emotionally neutral material.

A model of memory impairment in epilepsy cannot be concluded in these detail. Memory deficits in MTLE patients may vary in severity from mild subjective memory impairment to persistent amnesia that largely prevents the patient from learning any new information (Hermann et al 1997). The episodic memory, that is the explicit acquisition and later retrieval of new information, is particularly impaired. Obviously, this also leads to the impairment of remote (biographical) memory, although retrograde mnestic problems caused by seizures cannot be completely excluded (Bergin et al 2000). Such findings are in line with suggestions that the encoding of episodic information is the main problem in MTLE. However, associations of hippocampal MR spectroscopic imaging and confrontation naming indicate that temporomesial structures participate also in retrieval processes (Bell et al 2000; Sawrie et al 2000).

The left TL and right TL differentially contribute to material-specific memory, with the left hemisphere specialised in the processing of verbal material and the right hemisphere in processing of non-verbal, perceptual details or spatial attributes of material. In patients with MTLE, memory impairment tends to be material specific as a function of lateralisation of the epileptic focus (Helmstaedter et al 1997; Moscovitch et al 2002).

Mood and emotional processing

Further functional impairments probably related to temporomesial limbic structures refer to mood and social cognition. In MTLE patients, the incidence of depressive mood disorders is markedly increased, whereas multiple etiological mechanisms such as psychosocial, neurobiological and iatrogenic are suggested (Helmstaedter et al 2004; Kanner 2002). The question as to whether depression in MTLE depends on the side (left/right) or site of the lesion (mesial/lateral) is still under discussion. The data of Quiske et al. (2000) however suggest that impairment of temporomesial limbic structures (mostly MTL sclerosis) in contrast to focus of lateralisation appeared to be a predisposing factor for depressed mood.

The involvement of temporomesial limbic structures on more subtle impairments of social and emotional cognition have not been widely studied. Recent studies suggest right MTLE impairments in the ability to recognise fearful facial expressions (Benuzzi et al 2004; Meletti et al 2003), whereas patients with early onset of seizures were the most severely impaired. Subjects with lesions of the amygdala arising early in development demonstrated deficits in advanced tests of reasoning about the mental states of others such as detecting tactless or ironic comments or interpreting non-literal utterances (Shaw et al 2004).

3.1.3 Neurological diagnostics of MTLE

Mesial temporal lobe epilepsy is diagnosed by the history of characteristic partial seizure symptoms. The diagnosis is strongly supported by the electroencephalogram (EEG) and by the neuroimaging of a lesion.

Interictal EEG may provide valuable information in lateralising the seizure focus in patients with MTLE. The interictal EEG signature of this disorder are unilateral or bilaterally independent mesial temporal spike and spike-and-wave discharges. A single awake scalp EEG recording has a relatively low yield. The diagnosis is therefore confirmed by the capture of a typical episode during an EEG or video-EEG with epileptiform activity over one or both temporal regions (Devinsky 2004; Kilpatrick et al 2003).

Neuroimaging of anatomic lesions such as MTL sclerosis, are best revealed by high resolution magnetic resonance imaging (MRI). Magnet Resonance Imaging for MTL diagnostics relies on special MR-sequences, features suggestive of mesial temporal sclerosis. They include: T1 weighted volumetric acquisition to allow quantitative assessment of the hippocampal volume, T2 weighted acquisition and fluid attenuated inversion recovery (FLAIR) to detect increased signal in the hippocampus. Most important is that all coronal sequences shall be oriented orthogonal to the long axis of the hippocampus (Knowlton 2004). The concordance of visually diagnosed hippocampal atrophy and histologically verified cases is in experienced neuroradiologists around 80-90% (Koepp et al 2005). Quantitative MRI measurements such as volumetry or the definition of T2-relaxation times of the hippocampi are used to identify either subtle unilateral or bilateral hippocampal sclerosis in certain patients. These techniques increase the sensitivity to define hippocampal atrophy up to 90-95% (Koepp et al 2005).

Despite the use of high resolution images, MRI is normal in approximately 30% of patients with MTLE (Siegel et al 2001). In these patients greater emphasis is placed on intracranial EEG monitoring and functional imaging such as positron emission tomography (PET),

single photon emission tomography (SPECT) and MR-spectroscopy (Knowlton 2004). ^{18}F-fluorodeoxyglucose (FDG)-PET typically measures interictal hypometabolism, reflecting the preferential networks involved by ictal discharges and pathways of seizure spread (Koepp et al 2005). Specific PET ligands methods with FDG and [^{11}C]-flumazenil are used to identify the functional integrity of the GABAergic inhibitory neurotransmitter system (Koepp et al 2005). In studies, new PET ligands for opioid receptors, peripheral benzodiazepine receptors, serotonergic, cholinergic and glutamatergic neurotransmission have been tested in patients with MTLE and hippocampal sclerosis. They commonly give redundant information of reduced ligand binding in areas with atrophy. Ligand PET is unlikely to give better information than MRI in MTLE, but it could be used in the assessment of patients who do not have signs of hippocampal sclerosis on MRI (Koepp et al 2005). Ictal SPECT has developed into an established tool for aiding seizure localisation. It shows increased blood flow in the seizure originating side such as in the temporal lobe during complex-partial seizures in MTLE (Van Paesschen et al 2003). MR-spectroscopy is sensitive to detect bilateral pathology. Altered metabolism caused by neuronal loss or dysfunction and astrocytosis is indicated by ipsilateral reduction of N-acetylaspartate and increased choline-containing compounds (Petroff et al 2003).

Intracranial monitoring includes the implantation of subdural and/or depth electrodes for a period of days to weeks, to record seizure onset and to perform electrical stimulation mapping. This provides valuable information on the spatiotemporal dynamics of large neural assemblies that underlie pathology (Engel et al 2005). Morbidity from cortical stimulation mapping comes from several sources: bleeding during placement or withdrawal of electrodes, and local pathological changes corresponding to the length of time the electrodes were implanted (Dubeau et al 2000).

3.1.4 Therapy of MTLE

Mesial temporal lobe epilepsy is treated by medications or, when the seizures are refractory, by resective surgery. Half of patients respond to maximally tolerated doses of a single AED (Kwan et al 2001). If adverse effects do not permit the use of therapeutic doses, another monotherapy trial is usually tried (Kwan et al 2001). When seizures persist despite high plasma levels, a trial of two AEDs is recommended. When monotherapy fails, two AEDs will improve seizure control in more than one third of patients but will fully control seizures in only 10% (Mattson et al 1985).

Medications can be divided into older medications (called first-generation anticonvulsants such as phenytoin, carbamazepine, valproic acid or phenobarbital) and more recently

developed medications (second-generation anticonvulsants such as oxcarbazepine, vigabatrin, lamotrigine, zonisamide, gabapentin, tiagabine, topiramate and levetiracetam). Effectiveness and side-effects of the ‚older' AEDs are well established. Most of the new drugs are at least as effective as the 'older' AEDs but seem to be better tolerated.

Despite recent advances in the diagnosis and pharmacological treatment of localisation-related epilepsy, seizures remain refractory to medical therapy in approximately 30% of patients (Kwan et al 2000). For patients with pharmacoresistant seizures, epilepsy surgery has become a serious treatment option. Standard surgical techniques are selective amygdala-hippocampectomy and anterior temporal lobectomy with resection of the hippocampal formation, the amygdala, and, depending on the surgical approach, lateral temporal neocortex (Kim et al 2001).

Successful surgery is dependent on accurate localisation and lateralisation of the epileptogenic zone. The preoperative evaluation involves a series of assessments and investigations including detailed clinical history, interictal EEG, video-EEG monitoring, MRI, neuropsychology and neuropsychiatric assessments (Kilpatrick et al 2003). The findings of hippocampal sclerosis with the MRI techniques described above is highly predictive of a good outcome from surgery (Gilliam et al 2000). Approximately 70% of surgical candidates with MTLE become seizure free, and many others reach significantly reduced seizure burden and improved quality of life (Helmstaedter 2004).

3.1.5 Neuropsychological diagnostics and evaluation of therapy in MTLE

Because of the importance of cognitive symptoms in MTLE, neuropsychologists have a significant role in the assessment, treatment and rehabilitation of patients with MTLE. Disturbances of cognitive function is one of the major influence on quality of life in individuals with epilepsy (Trimble 1994) and may be more debilitating than the seizures itself (Rausch et al 1997). Cognitive impairments result from various interacting factors such as aetiology, age of onset, type of epilepsy, type of seizures, seizure frequency, seizure duration, seizure severity, medication and duration of epilepsy (Jokeit et al 2004).

Neuropsychological diagnostics aim at determining the mental status including information of individual's cognitive strengths and weaknesses, to examine epilepsy- or lesion-related cognitive impairments in order to yield diagnostic information that helps in lateralising and localising the seizure focus, and finally to control for quality of treatment effects, namely drug and surgery, on patients' cognition (Baker et al 2004; Cull et al 1997). Each of these specific roles require careful consideration with respect to the potential pitfalls they can present.

Cognitive side-effects of AEDs

All commonly used AEDs have some effects on cognitive functions, whereas individual reactions to the cognitive effects of AEDs vary. Some patients experience problems even with low serum levels, whereas others tolerate high levels with few or no complaints (Devinsky 1995). In general, severity of cognitive side effects are considered to be mild to moderate for most AEDs (Vermeulen et al 1995). But the impact can be amplified in specific conditions and may become substantial in some patients when crucial functions are involved or when functions are impaired that are already vulnerable such as memory function in the elderly (Trimble 1987).

All of the older AEDs (i.e. valproic acid, carbamazepine or phenytoin) have ‚absolute' cognitive side effects, meaning that all of the investigated drugs have cognitive effects when compared with no treatment in the same subjects (Aldenkamp et al 1987; Aldenkamp et al 2003). All AEDs are reported to have adverse effects related to dosage (Devinsky 1995) and are stronger for polypharmacy compared with monotherapy (Aldenkamp et al 2003; Trimble 1987). The new AEDs might have less influence on cognitive functions but there are only a few controlled studies available (Brunbech et al 2002).

The most prevalent of the cognitive side effects observed in an AED therapy are impairments in attention, psychomotor and mental speed. Focal deteriorations are less frequent and have been observed for memory in phenytoin (Aldenkamp 2001) and carbamazepine (Meador et al 1993; Meador et al 1991) as well as for verbal skills in topiramate (Aldenkamp et al 2003).

Regarding memory, the data are not finally conclusive, because recent studies lacked to adequately control for seizures, interictal discharges and underlying brain disease (Motamedi et al 2004). The adverse effects of AEDs on memory in patients with epilepsy may be partly offset, in part, by reductions in seizures and interictal discharges. Treating the seizures with AEDs may have positive effects on memory performance such as observed in animal models. For example, studies in rats trained to run from electric shock into a maze have shown better memory performance in normal rats treated with placebo than in those treated with carbamazepine. On the other hand, epileptic rats treated with carbamazepine functioned better compared with those who were not treated (Hawkins et al 1985).

In addition, the effects of AEDs may interact with focal brain dysfunction. For example, patients with MTLE were tested on a word list learning paradigm under conditions of high

and low AED dosages (Durwen et al 1993). Memory performance on this verbal task improved with AED dose reduction, but only for left MTLE patients and not for right MTLE patients. The significant group differences in verbal memory for the left and right MTLE groups under full AED dose disappeared with dose reduction.

Another factor affecting the results is mood. Mood alters subjective perception of memory, but it is unclear what effect mood has on objective memory performance in epilepsy patients and what interactive effects AEDs have on mood and memory (Motamedi et al 2004).

Cognitive side-effects of TLE surgery

Following TLE surgery, potential increases and selective decreases in cognitive functions are likely to occur. Improvements following TL surgery are mostly related at least in part to successful seizure control. When patients become permanently seizure-free, a hierarchical release or recovery of extratemporal functions can be observed within the first year of surgery which is followed by improvement of TL functions in the longer follow-up (Helmstaedter et al 2002). On the other hand, patients undergoing TLE surgery are at risk for memory deficits (Gleissner et al 2002).

Patients undergoing left TLE surgery are generally considered to be at greater risk for postoperative memory decline than those undergoing right TLE surgery. In general, the degree of memory decline depends upon both the functional status of the ipsilateral MTL that will be included in the resection and on the functional status of the MTL contralateral to surgery which will be relied upon for the formation of new memory.

Patients undergoing TL surgery are at risk for postoperative severe amnesia if the MTL contralateral to the surgery is diseased and non-functional (Loring et al 2001a). The most famous patient is H.M. (Scoville et al 1957), who suffered from a severe amnesic syndrome after surgical resection of both temporal lobes. H.M. was unable to acquire any new information for later recall, whereas memory performance of immediate memory and skill learning remained intact (Corkin 2002). The very few publications on amnesic patients since the publication of the patient H.M. indicate that amnesia can hardly be predicted, or that it is a late rather than an acute consequence in patients who decompensated after unilateral temporal lobectomy due to contralateral damage after status epilepticus, severe seizures, or head injury (Dietl et al 2004; Oxbury et al 1997). A recent review shows two of seven cases in whom asymmetry in memory performance

might have been predictive for amnesia but this study does not show how often the same pattern is seen without the severe consequence of amnesia (Kapur et al 2003).

To summarise, postoperative losses following TL surgery can be reduced when surgery is restricted to tissues not any more involved in function. Additionally, a better outcome is observed with greater cerebral plasticity and greater capacities for compensation. Regarding reserve capacity, the major determinants of a better cognitive outcome after TL surgery are surgeries within the non-language-dominant hemisphere, younger age at the time of surgery, better baseline performance, better intellectual capacity, and more successful seizure control (Helmstaedter 2004).

The impact of neuropsychological diagnostics is therefore to anticipate a potential risk for postoperative memory dysfunction. Besides the traditional neuropsychological examination, the intracarotid amobarbital test (IAT) and increasingly also fMRI is used to define presurgically the eloquent cortex. The method of fMRI will be discussed in detail in the next chapter. The IAT procedure is currently the most common and reliable method used to assess language and memory function (Loring et al 2001b). The IAT anaesthetises an entire hemisphere in order to test the other hemisphere for language and memory function, including verbal and visual encoding or retrieval efficiency. It is an invasive procedure, difficult to repeat and is, unfortunately, associated with rare but significant complications.

Beyond the function of MTL structures in memory, the amygdala is one of the key structures involved in emotional processing. Patients undergoing TL surgery may therefore be at risk for impairments in emotional processing, including social behaviour and specific functions of social and emotional cognition (Adolphs et al 2001; Anderson et al 2000; Brierley et al 2004; Hermann 2002). However, the question of whether certain impairments following TL surgery are related to resected parenchyma of the amygdala is a matter of controversy.

3.2 Functional MRI in MTLE

Functional MRI plays an increasing role in the diagnostics of MTLE. Moreover, the use of fMRI in presurgical evaluation of patients with epilepsy is probably the most important clinical application (Vingerhoets et al 2004).

Functional MRI relies on blood oxygen level dependent contrasts to detect cortical areas of neuronal activation. In epilepsy diagnostics it is mainly used to map language and memory functions. It also contributes to the detection of seizure lateralisation, because

memory functions are subserved by the same brain regions that typically harbour the seizure focus itself in TLE (Detre 2004). Functional MRI is not yet established in other areas of neuropsychological interest, e.g. effects of AEDs on cognition.

Generally, the use of fMRI in a clinical setting presents challenges beyond those encountered in studies with healthy volunteers. Functional MRI relies on very small regional changes. In single-case designs such as in a clinical setting and especially within the MTL, the signal changes are even smaller. The main demand is therefore to design fMRI applications which yield strong, robust and meaningful results on single-subject level.

3.2.1 General considerations on the use of fMRI in MTLE

Before this report will turn to the topic of the clinical application of fMRI in MTLE, a short presentation of the fMRI-technique and specific areas for its clinical use will be provided.

3.2.1.1 Functional MRI techniques and the neural basis of the BOLD signal

Functional MRI is not a direct measure of neural activity. Instead, signal intensity alterations are caused by stimulus-induced local changes in blood oxygenation, called the blood-oxygen-level-dependent (BOLD)-contrast. It is based on the differing magnetic properties of oxygenated (diamagnetic) and deoxygenated (paramagnetic) blood. These differences in magnetic susceptibility lead to small, but detectable changes in susceptibility-weighted MR image intensity.

A typical BOLD response consists of a 1-5% change in regional image intensity which develops over a 3-8s period after task initiation, typically with an initial peak or overshoot, a somewhat lower plateau for sustained tasks, and often an undershoot of the baseline after task completion (figure 1, white line). The peak latency of several seconds represents a major limiting factor in the temporal resolution of functional imaging methods (Hennig et al 2003).

The relationship between neuronal activity and fMRI signal is quite complicated, involving multiple neurophysiological mechanisms such as interaction between blood flow, blood volume and haemoglobin oxygenation. Functional BOLD contrast is obtained because the iron, present in haemoglobin, becomes paramagnetic only when it is deoxygenated, producing a local susceptibility increase (Ogawa et al 1993). With regional brain activation, decreases in regional deoxyhaemoglobin occurs which has been attributed to increases in cerebral blood flow (CBF) and glucose consumption that exceed increases in oxygen consumption. The precise basis for this mismatch is uncertain, but recent studies

have shed light onto both the cellular mechanism of metabolic and vascular processes that are relevant to functional imaging and the neuropyhsiological underpinnings of the fMRI signal.

The basic principle is that neuronal activity is closely connected to changes in blood flow and oxygenation and energy metabolism. Recent data suggests that both, the vascular and the metabolic response to neuronal activity are mediated by glutamatergic neurotransmission (Attwell et al 2002; Magistretti et al 1999). Glutamate is the main excitatory neurotransmitter in the brain and is released following neuronal activation. After it is released, glutamate needs to be removed promptly from the synapse and this occurs by uptake into an adjacent non-neuronal cell - an astrocyte. There, it is converted to glutamine before it can be returned to the neuron where it is recycled. The neurometabolic coupling to glutamatergic neural activity is based on the fact that all steps from the release of glutamate to its transformation as well as its actions on postsynaptic receptors require energy. This energy demand is preferentially met by the glycolytical transformation of glucose into lactate in astrocytes and by oxidative phosphorylation of glucose and lactate in the neuron. Both pathways contribute significantly to glucose consumption (Magistretti et al 1999).

Neurovascular coupling to glutamatergic neural activity is explained by the release of nitric oxide and prostaglandin from neurons or astrocytes upon a glutamate invoked intracellular rise in calcium. Nitric oxide and prostaglandin are potent vasodilator agents and thus leading to an increase in blood flow during activation. Although, neurometabolic and neurovascular coupling are both mediated by glutamatergic transmission, the increase in blood flow and energy utilisation can be dissociated and should be considered to the results of processes operating in parallel.

The neurophysiological basis that underlies the BOLD signal has been investigated by simultaneously recording neuronal activity and fMRI. A number of studies have demonstrated that the haemodynamic response reflects directly an increase in neural activity elicited by a stimulus (Logothetis et al 2001; Ogawa et al 2000; Rees et al 2000). BOLD responses and neural responses even seem to have a linear relationship for stimulus presentations of short duration. Logothetis and co-workers (2001) simultaneously recorded in their experiments electrophysiological signals and fMRI responses in anaesthetised monkeys that were viewing checkerboard patterns. The authors have shown that the BOLD contrast directly reflects neural responses elicited by a stimulus and that BOLD signal and neural activity are linearly correlated for short stimulus presentation duration.

3.2.1.2 The experimental design

During an fMRI scan, a task is presented to the subject while lying in the scanner. It requires the subject to perform specific acts and thereby induces series and complexes of neuronal events in a controlled manner. Task-specific BOLD signal changes are not directly quantifiable in physiological units. They are typically measured relative to a baseline condition and expressed as a percentage signal change or as a statistical significance level based on a particular statistical model.

The traditional approach of conducting an fMRI study is the so called block design (figure 1). Block designs encompass alternating epochs of task and control conditions. This approach maximises sensitivity, because large signal changes are sustained, and also minimises dependence on an accurate estimate of the activation-flow coupling response.

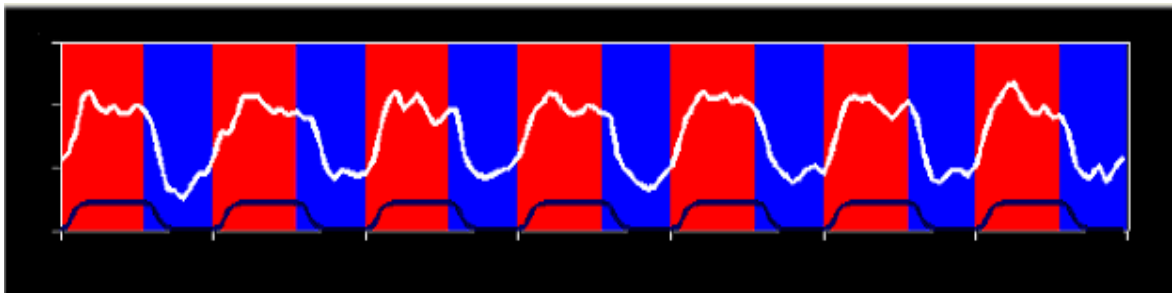

Figure 1: Outline of stimulus presentation and response for a simple functional magnetic resonance imaging (fMRI) experiment in primary visual cortex. The boxcar fMRI stimulus presentation paradigm consists of alternating periods of stimulation (on-state, red) and rest (off-state, blue). A correlation waveform (black) is used for analysis. It is delayed slightly with respect to the stimulus presentation in order to allow for the lag between neuronal activity and the haemodynamic response. The white line represents an example of raw data set.

It is assumed that subtraction of images acquired during an ‘on-state’ and an ‘off-state’ can be used to isolate activation responses. For example, comparison of a visual stimulation (on state or active state) to darkness (off-state or control state) activates primary visual areas.

Many brain functions, such as memory cannot be isolated that way, because they are coupled to other functions (Friston et al 1996), including attention, language or executive functions. More complex sets of tasks are required, involving multiple task-demands. The activity is then averaged over different cognitive processes that can occur during a specific task (Maguire et al 2003).

Because the time course of the haemodynamic response measured with the BOLD method is slow relative to the primary neuronal activation event, the rates of changes

between cognitive states in a paradigm must in most cases be kept slow relative to the expected time course of the haemodynamic response. The optimal duration of an epoch is determined by the time course of the haemodynamic response and typically lasts from 16s to 30s.

Another experimental approach is the so called event-related fMRI experiment. In contrast to the block-design, it allows to sort and to average the brain activity of events based on participants' responses such as whether a stimulus was recognised or not in a memory task. However, the signal detection power of event-related fMRI is much lower than that in a block design (Liu et al 2004). Even more, the MTL is a non-optimal target for event-related fMRI, because the magnitude of the signal is much lower (less than 1% (Powell et al 2004)) than in primary sensory areas. Thus, event-related fMRI is a valuable tool for the measurement of perceptual functions, in which a high number of stimuli can be presented and the loss of signal detection power can be afforded, in contrast to MTL-related structures and single-case studies such as in clinical measurements, in which these limitations can lead to serious disadvantages.

The challenge to design fMRI-paradigms in a clinical setting is to develop tasks which on the one hand activate reliably and robustly the target structure and which have on the other hand a low cognitive demand, so that patients can do them. Nevertheless, they have to be interesting, so that patients cooperate well. Scans in which there is little or no activation might suggest poor cooperation, sleep or an inability to do the task.

3.2.1.3 The difficulty of imaging the MTL in clinical studies

The signal within the MTL is low. The TL is most confronted with high susceptibility artefacts caused by inhomogenities due to the differing magnetic properties of bone, tissue and air in its vicinity (Powell et al 2004). The TL is therefore especially likely to suffer from geometric distortions or loss of BOLD signal (Jezzard et al 1999). The observation that multisubject group maps tend to demonstrate much more robust activation in the hippocampal head than individual subject studies (Killgore et al 1999) provides further evidence suggesting that detecting this activation is limited primarily by sensitivity. However, averages of activation patterns are not necessarily representative for each individual subject especially in clinical studies and therefore meaningful data at the single-subject level is required.

On the technical side, a number of recent developments in MRI hardware might greatly increase the sensitivity of fMRI for MTL activation. Recent efforts to overcome the problems include techniques such as z-shimming (Rabin et al 2004) or ultra-thin slices

(Constable et al 1993) to minimise susceptibility artefacts. Another approach is the use of higher field strength magnets such as 3T or even 7T. The use of higher magnetic fields improves contrast and elevates sensitivity to capillary level blood flow changes coupled with neuronal activity (Hennig et al 2003; Krasnow et al 2003). A problem is that higher fields require faster 'gradient switching' (i.e. the additional magnets that are switched as part of the data acquisition scheme), resulting in higher levels of electromagnetic power and subsequently in the risk of inducing nerve stimulation. Unfortunately, high magnetic fields also magnify some of the problems inherent in MRI such as field distortions and motion artefacts (Hennig et al 2003). One of the main concerns in clinical use of fMRI are the artefacts and noise from motion, since patient movements of the order of a few millimetres during the imaging can severely deteriorate the quality of the data and induce severe artefacts (Koepp et al 2005). Clinical fMRI-studies on higher field strength such as 3T is therefore difficult. It is not surprising that only one study is available demonstrating the possibility of imaging epilepsy patients at 3T (Szaflarski et al 2004). As the study had a small sample size (no more than six patients), the data are preliminary and the utility of high field fMRI in a clinical context needs to be investigated in a larger population. However, 1.5T is the norm for clinical scanners, at least within the next years. Therefore other approaches than higher field strengths have to be discussed to increase the signal-to-noise ratio (SNR). Unrelated to the MTL, several attempts have recently been undertaken to enhance BOLD signal intensity with the application of pharmacological substrates. The studies are related to primary sensory and motor areas, but it might be hypothesised that the MTL could profit by a pharmacologically induced contrast booster, too. The application of methylxanthines such as theophylline or caffeine has been recommended as a BOLD contrast booster for studies confronted with low SNR (Laurienti et al 2002; Mulderink et al 2002). However, the studies presented above used group-results which are not necessarily representative for clinical applications. In addition, the research of caffeine as a BOLD contrast booster is restricted to primary sensory and motor modalities (Laurienti et al 2002; Mulderink et al 2002), whereas the effects on the MTL have not yet been investigated. The first study of the present thesis investigates therefore whether caffeine is an effective BOLD contrast booster within MTL for clinical studies.

3.2.1.4 The importance of reproducibility for clinical studies

Because of the low SNR in MTL, and especially in single-case studies, it is indispensable to validate clinical fMRI paradigms in terms of its retest-reproducibility. The requirements to validate the reliability of activation patterns is highest in presurgical investigations,

because the results have a clear and significant impact on patients' health. In the context of presurgical mapping for example, it has to be proven that single-session results are valid and it must be demonstrated that the patterns of brain activation developing in response to the same task are repeatable between imaging sessions.

In spite its importance, there are only few studies concerned with the reproducibility of single-session fMRI protocols. Additionally, the studies might be biased because of underrepresentation of negative findings in the peer reviewed literature. However, in the available studies, a relatively high consistency of activation pattern over time has been reported, mainly in motor or visual areas (Rombouts et al 1998; Yetkin et al 1996), but also for cognitive tasks such as working memory (Wei et al 2004), visual encoding (Machielsen et al 2000) and episodic retrieval (Miller et al 2002). Regarding memory-related structures Miller and co-workers (2002) observed highly variable activation patterns from individual to individual. Despite large variations from subject to subject, those individual patterns of activation were reliable over time. The authors suggest that individuals may have been using very different strategies and cognitive processes during the task, and those differences were reflected by different patterns of brain activations.

Some clinical questions demand the possibility to evaluate the quantitative reproducibility of the BOLD response in serial investigations. Reproducibility of activation magnitude as a quantitative approach is desired to investigate any changes within a given structure and reports the reproducibility in terms of the magnitude and spatial extent of activation. This is difficult to obtain, because there are a lot of factors affecting the BOLD response such as technical (instability of the scanner, drifts of the man magnetic field during the scanning session), physiological (e.g. hyperventilation) or psychological phenomena (e.g. attention effort (Specht et al 2003)). The evaluation of quantitative reproducibility is far less used in clinical settings than the reproducibility of activation patterns.

Unfortunately, quantitative reproducibility seems to be poor on intra-individual levels (McGonigle et al 2000; Miki et al 2000; Moser et al 1996; Waldvogel et al 2000). At least, lower variability within subjects than between subjects has been reported (Aguirre et al 1998; Wei et al 2004). But the reported studies rely mainly on primary visual and motor areas, whereas cognitive tasks are scarcely investigated (Machielsen et al 2000). It is therefore questionable whether cognitive fMRI tasks lead to stable activations. Since Specht and co-workers (2003) reported increased reliability in attended compared to unattended visual tasks, it might be suggested that cognitive tasks with higher complexity and more task demands reveal higher retest-reliability.

3.2.2 Functional MRI in the evaluation of cognitive side-effects of AEDs

To date, neuropsychological testing has been the major method of objectively examining cognitive functions related to the use of AEDs, but methodological inaccuracies in clinical studies may obscure the effects of AEDs on cognition. As already discussed, it is difficult to control for interactions of AEDs with seizures, interictal discharges, underlying brain disease and mood. Therefore, innovative methods such as fMRI may help to avoid some of the confounds.

Besides the field of epilepsy, fMRI has widely been administered to investigate drug effects. Effects on the magnitude or extent of the percent BOLD signal change have been observed in both directions such as caffeine boosted the BOLD signal (Mulderink et al 2002) while ethanol had inhibiting effects (Levin et al 1998; Seifritz et al 2000). Common paradigms are visual and motor tasks. Studies regarding memory are few, such as Sperling and co-workers (2002) who observed a correlation between scopolamine and lorazepam-induced memory deficits and reduced activation in the hippocampus and fusiform gyrus.

Regarding study designs, only few studies point out significant intra-individual drug induced effects (Loubinoux et al 2002; Martin et al 2000; Sell et al 1997). Exactly single-case designs would be crucial in the investigation of drug effects in epilepsy patients. Methodologically, it has to be considered that few studies (e.g. Loubinoux et al 2002; Rao et al 2000; Seifritz et al 2000) used well defined conditions and provided reliability estimations. The latter are inevitable because the measurements are administered under different drug-conditions and the BOLD response is already variable over repeated scans (Machielsen et al 2000; McGonigle et al 2000; Waldvogel et al 2000).

Although fMRI has increasingly been applied in neuropharmacological studies dealing with drug effects on the brain (Honey et al 2004; Leslie et al 2000), investigations about the effects of AEDs on the BOLD response are rare. There are only two studies dealing with this topic. Bell and co-workers (2005) investigated the effect of dextroamphetamine on the brain in valproic acid treated subjects. The pre-treatment with valproic acid led to decreased signal-changes in a working memory task. Another study found a correlation between carbamazepine serum level and decrease in the memory induced activation of MTL structures (Jokeit et al 2001b). Memory-related MTL activation was induced by the Roland's Hometown Walking Task (Jokeit et al 2001c) which encompasses mental navigation and recall of landmarks of a familiar visuospatial environment. The observed decrease is consistent with reports of behavioural memory decline on high doses of carbamazepine (Meador et al 1993) and with reduced cerebral glucose metabolism as

shown by glucose PET in epilepsy patients receiving carbamazepine (Theodore 1988). However, it is necessary to replicate this result in longitudinal studies, ideally covering initiation, chronic administration and withdrawal of AED.

3.2.3 Functional MRI in presurgical MTLE diagnostics

Presurgical evaluation of MTLE patients using fMRI includes the mapping of language and memory. With the introduction of fMRI it was hoped that this new technique might supersede invasive procedures such as the IAT or cortical stimulation. In contrast to such invasive tests, fMRI is less risky, repeatable and more widely available. But, before non-invasive fMRI can replace them, it is necessary to show that first fMRI can be used in the majority of patients otherwise undergoing invasive procedures that secondly fMRI acquisition and assessment are easy and reproducible and third that fMRI provides typical and atypical results in large numbers of individual cases in concordance with a standard in presurgical diagnostics (e.g. the IAT) or postoperative outcome (Hammeke et al 2000).

Language

Within presurgical diagnostics of MTLE patients, language lateralisation is the most widely studied clinical application of fMRI. Tasks are related to semantic judgement, verbal fluency, story comprehension and combined tasks (Detre 2004). By the use of comprehensively designed series of language tasks, fMRI has been able to reliably identify the language-dominant hemisphere, to provide estimates of localisation of activated networks and to identify eloquent language regions in the vicinity of the surgical target, thus reducing the risk of serious postoperative complications (Matthews et al 2003).

The external validation with presurgical invasive methods has best been shown for language-lateralisation, where a high concordance of 90% between fMRI and IAT was found, with atypical lateralisation predicting postsurgical deficits. In left-sided TLE, the rate of false categorisation by fMRI was not higher than 3%, whereas the rate of false categorisation increased to 25% in left-sided extratemporal epilepsy (Binder et al 1996; Sabsevitz et al 2003; Woermann et al 2003).

The information about language dominance is also important for subsequent evaluation of memory functions, because it can significantly influence its laterality. For instance, in patients who have atypical language dominance due to early epileptogenic lesions in the left hemisphere, re-allocation of language and verbal memory to the right hemisphere can

cause deficits of functions that are originally lateralised to the right hemisphere (Akanuma et al 2003). The presence of atypical language dominance is higher in epileptic patients than in the general population. 33% of patients with left-sided intractable temporal-lobe epilepsy show atypical language representation with bilateral or right hemisphere language-related lateralisation (Adcock et al 2003).

Memory

Results from memory-related fMRI measurements are of use in determining the risks of amnesic complications from temporal surgery. Additionally, memory-activation patterns observed with fMRI also contribute to seizure lateralisation, because memory functions are subserved by the same brain regions, where the seizures typically arise in TLE (Detre 2004).

The utility of fMRI for lateralising memory function in presurgical testing of medically refractory MTLE patients has been shown in recent studies by using episodic encoding memory paradigm with complex visual scenes (Detre et al 1998; Stern et al 1996) or multimodal stimuli (Golby et al 2002; Kelley et al 1998) or by retrieval from longterm-memory (Jokeit et al 2001c). The principle of memory fMRI-paradigms bases on the aim to bilaterally activate the MTL during memory tasks, because fMRI is not capable of assessing the unilateral capacity of the MTL structures thought to be critical in forming new explicit memory. Accordingly, the failure to activate the left or right MTL reflects impaired function. Jokeit and co-workers (2001c) have shown that their fMRI memory paradigm, the Roland's Hometown Walk, effectively lateralises the epileptic temporal lobe by activation of the parahippocampal gyri. The advantage of this paradigm is the retrieval from longterm-memory which is very close to the patients' everyday practical use of their memory.

Compared to language-lateralisation, less meaningful data regarding external validation of memory-related fMRI with IAT are available. The studies rely on small sample sizes, mostly with left-sided MTLE cases (Detre et al 1998; Golby et al 2001; Rabin et al 2004). Nevertheless, the results are promising as far as positive correlations were found between presurgical activation asymmetry ratio within MTL and hemispheric memory dominance found with IAT (Detre et al 1998; Golby et al 2001; Rabin et al 2004). The Roland's Hometown Walking Task finally, correctly lateralised the side of seizure onset in 90% of 30 patients with unilateral MTLE (Jokeit et al 2001c).

It has therefore to be mentioned that although fMRI of memory-lateralisation is far from widely replacing the invasive IAT, it even now contributes to seizure lateralisation and determinates the risks of amnesic complications from temporal lobectomy (Detre 2004). Regarding the latter, recent studies suggest a predictive value of fMRI for postsurgical memory outcome (Koepp et al 2005). Rabin and co-workers (2004) used a complex visual scene-encoding task to show a relation between MTL activation asymmetry ratios and postsurgical memory outcome; increased activation ipsilateral to the seizure focus was related to greater memory decline. In an event-related linguistic encoding fMRI study in patients with left hippocampal sclerosis, greater activity in the left hippocampus compared with the right hippocampus predicted the extent of verbal memory decline after left anterior temporal lobectomy (Richardson et al 2004). Using the Roland's Hometown Walking Task, a recent study (Janszky et al 2005) showed that this task can predict postoperative memory decline in MTLE. Memory-fMRI findings correlated with individual memory decline measured by a non-verbal memory test after right-sided anterior temporal lobectomy.

The combination of presurgical information including memory-related fMRI results, structural MRI with information about the hippocampal volume and neuropsychological assessment improve the accuracy of prognosis for cognition made before unilateral anterior temporal-lobe resection. Surgical procedures could be modified in those patients with a high risk of cognitive impairment and the preoperative patient counselling could be improved (Koepp et al 2005).

Other functions related to MTL

Besides the presurgical diagnostics of memory-related MTL structures and in contrast to the invasive IAT, fMRI would provide the possibility of mapping additional features such as the amygdala. In recent presurgical fMRI-protocols of MTL structures, the amygdala has rarely been evaluated. Imaging the amygdala presurgically might allow one to evaluate the possible clinical implications for amygdala-related functions residing within the to be resected tissue and may help to identify candidates who are at risk of emotional and social impairment following TL surgery.

4 General questions

One of the major concerns of neuropsychological diagnostics of MTLE is to evaluate and to prevent negative treatment effects on patients' cognitive profiles. Both, pharmaco-therapy and epilepsy surgery may have substantial influence on cognition. The fact that cognitive disturbances are one of the major influence to impair quality of life in individuals with epilepsy (Trimble 1994) makes the investigation of such treatment effects necessary.

In addition to the classical neuropsychological examination, fMRI is increasingly being applied in clinical diagnostics. The most important and the most advanced clinical application of functional MRI is found in presurgical diagnostics, whereas investigations of pharmacological treatment effects are few and rely on group and cross-sectional studies.

The studies reported here address three selected topics in the field of clinical fMRI within the MTL. The first study approaches a more technical question of fMRI within MTL, namely whether certain substances are able to increase the low BOLD contrast in this region which is confronted with low SNR. The second and third study take up recent advances of fMRI in pharmacological fMRI and in presurgical diagnostics.

In all three studies we used the Roland's Hometown Walking Task (Jokeit et al 2001b) to activate the MTL. This task uses a block-design. During the activation block, the subjects have to mentally navigate through their hometown and to retrieve spatio-temporal information from long-term memory. The baseline condition consists of covertly counting odd numbers, starting with 21. This paradigm has been shown in many studies to reliably activate the MTL bilaterally on a single-subject level, including patients with MTLE, patients with low formal IQ, children, older subjects and subjects from other cultures (Avila et al 2004; Janszky et al 2005; Janszky et al 2004; Jokeit et al 2001b; Jokeit et al 2001c). The advantage of this paradigm is its practicability, reliability and its close relation to every-day memory demands. A disadvantage of the paradigm is that there is no control of memory performance. We therefore added a behavioural control task and modified the task as follows: In preparation for fMRI acquisition, the subjects had to fill out a familiarity questionnaire of the landmarks of their hometown. Only landmarks with high familiarity were included in the individual hometown walks. Blocks with approximate ways (including similar type and similar number of landmarks) of approximately 300 meters were included. To control for memory performance during the fMRI-task, the subject was asked to write down the starting and endpoint of the 10 walks after each fMRI-session.

4.1 First study: Caffeine study

4.1.1 Aim of the first study

Functional MRI bases on very small regional signal intensity changes. One of the major difficulty in the application of fMRI in a clinical context is therefore to yield meaningful results on single-subject level. The use of a contrast booster would simplify fMRI measurements in clinical contexts, especially within the MTL, where the SNR is genuinely low. Recently, the application of caffeine has been recommended as a BOLD contrast booster for studies confronted with low SNR. However, the reported studies were restricted to primary sensory and motor modalities (Laurienti et al 2002; Mulderink et al 2002).

This study was aimed at evaluating caffeine as a contrast booster for fMRI-investigations in a clinical context. We intended therefore to reproduce the reported group-results of an increased percent BOLD signal change following caffeine intake in a single case, placebo controlled, repeated fMRI study within three distinct domains, namely, within primary visual and motor area and within memory-related MTL-structures.

4.1.2 General questions

(1) Are the reported group results of caffeine as a BOLD contrast booster within primary visual and primary motor area reproducible on the single-subject level?

(2) Does caffeine enhance the BOLD contrast also within the MTL and are these enhancing effects observable on the single-subject level?

4.1.3 Hypotheses

Recently, Mulderink and co-workers (2002) suggested the use of caffeine as a BOLD contrast booster for studies confronted with a low SNR. The authors reported a considerable overall increase of 26-37% in the BOLD-contrast in a visual (V1) and a motor (M1) area during the performance of a visually cued motor task after application of 200mg caffeine. In addition, Laurienti et al. (2002) found an increased BOLD signal in the visual cortex induced by visual stimulation following 250mg caffeine intake in high caffeine users. These studies referred to group designs without administration of both placebo and caffeine conditions (Mulderink et al 2002) or pre- and post caffeine conditions in one single subject (Laurienti et al 2002). In contrast, we chose an extended study design with

each person acting as their own control in four baseline, two placebo and two caffeine measurements.

(1) We hypothesise that the application of caffeine is efficient to boost the BOLD contrast on a single-subject level.

Although highly desired, there is no research into the use of BOLD contrast boosters such as caffeine within the MTL. It might be expected that caffeine enhances the BOLD signal within the MTL as it was observed within primary visual and motor areas. Caffeine is a non-selective adenosine receptor antagonist and blocks neurovascular receptors with a slightly higher affinity than that of neural receptors (Dunwiddie et al 2001). Blockade of vascular adenosine receptors produces vasoconstriction and decreases resting cerebral perfusion (Dunwiddie et al 2001). This decrease in baseline cerebral perfusion might produce an enhanced BOLD signal intensity (Dager et al 2000; Mulderink et al 2002). It might be suggested that this mechanism is not restricted to special regions such as primary visual and primary motor areas and that therefore an enhanced BOLD signal following caffeine intake might be observed within the MTL, too.

(2) We hypothesise that caffeine enhances the BOLD contrast within the MTL and that enhancing effects can be observed on a single-subject level.

4.2 Second study: Reproducibility of serial fMRI scans within the MTL

4.2.1 Aim of the second study

Antiepileptic drugs may have mild to moderate cognitive side effects, but their impact may be substantial when critical functions such as memory in MTLE patients, are involved (Aldenkamp 2001; Kwan et al 2001). Therefore, the cognitive side-effects of AEDs on memory-related MTL structures needs to be evaluated.

To date, neuropsychological testing has been the major method of objectively examining cognitive side-effects related to the use of AEDs. However, it allows no control for interactions of AEDs with seizures, interictal discharges, underlying brain disease and mood. Innovative methods such as fMRI may help to overcome some of the limits of the classical neuropsychological examination.

Besides the field of epilepsy, fMRI has increasingly been applied in neuropharmacological studies dealing with drug effects on the brain (Honey et al 2004; Leslie et al 2000). But recent studies mainly addressed short-acting drug effects, cross-sectional designs and group analyses. AEDs on the other hand are long-lasting treatments which require longitudinal study designs. Because of the heterogeneity of epilepsy patients, investigations on a single-subject level would be desirable.

The present study was therefore aimed at investigating the longterm-reproducibility of serial fMRI scans within the MTL. We used the Roland's Hometown Walking Task (Jokeit et al 2001c) to activate memory-related MTL structures. We investigated whether this task is robust enough to investigate the cognitive side-effects of AEDs in future studies. It was aimed at describing the variability of fMRI results with respect to number of significantly activated voxels and percent BOLD signal change on single-subject and on group level. The knowledge about the amount of variability is indispensable to distinguish between the variations inherent in the examination method and the treatment induced changes in subjects' brain activation in future studies.

4.2.2 General questions

(1) How variable are the results (number of significantly activated voxels and percent BOLD signal change) of serial fMRI scans elicited by the Roland's Hometown Walking Task within the MTL in single-subject and in group analyses?

(2) Does the observed variability of the fMRI-results (number of significantly activated voxels and percent BOLD signal change) allow future investigations of treatment effects of AEDs on the MTL?

4.2.3 Explorations

The longterm-reproducibility of fMRI results with more than three scans has rarely been investigated. On group analyses, Wei and co-workers (2004) recently reported small longitudinal variability in a working-memory task over nine serial fMRI-scans. The intersession variability of serial scans on the single-subject level has been observed in two studies (McGonigle et al 2000; Waldvogel et al 2000). Both found high intra-individual variability. There is no single study reporting quantitative retest-reproducibility of serial fMRI scans.

(1) We will explore the magnitude of variability of MTL activations induced by the Roland's Hometown Walking Task over serial scans in single-subjects and in group analyses.

The knowledge about the magnitude of variability is indispensable to distinguish between the variations inherent in the examination method and the changes in subject's brain activation by treatment effects in future studies. Only two pharmacological fMRI studies investigated drug-effects within the MTL. Both, the anticholinergic drugs lorazepam and scopolamine (Sperling et al 2002) and the antiepileptic drug carbamazepine (Jokeit et al 2001b) decreased the BOLD signal within the MTL. Compared to a placebo-condition, lorazepam and scopolamine decreased the number of significantly activated voxels within the hippocampus by approximately 40-60% and the percent BOLD signal change by approximately 50-60%. Including only high doses of carbamazepine (serum levels of more than 7 mg/l), the decrease of significantly activated voxels within the MTL was around 50%. Whenever side-effects of a pharmacological agent shall be detected with fMRI, the amount of variability of the used method has to be below those anticipated drug effects.

(2) We will explore whether the magnitude of the observed variability will allow future investigations of treatment effects of AEDs on the MTL.

4.3 Third study: Amygdala fMRI in MTLE

4.3.1 Aim of the third study

Functional MRI is an important method in presurgical diagnostics of patients with refractory MTLE. In the past, fMRI diagnostics of MTLE concentrated on the evaluation of memory-related structures including the hippocampus and parahippocampus, whereas the amygdala was rarely evaluated. Jokeit and co-workers (2001c) have shown earlier that the Roland's Hometown Walking task effectively lateralises the side of MTLE by memory-related activation of the parahippocampal gyri. Furthermore, the paradigm has been validated with the intracarotid amobarbital test (Woermann et al 2003) and the postsurgical outcome (Janszky et al 2005).

The present study is aimed at providing an fMRI paradigm to investigate the amygdala. We developed an fMRI paradigm that includes the visual presentation of sequences from thriller and horror movies showing actors portraying fearful faces. With the use of two distinctive fMRI-paradigms, mapping both the amygdala and the parahippocampus, it is aimed at improving the reliability of MTL diagnostics in MTLE patients.

4.3.2 General questions

(1) Is it feasible to image the amygdala reliably in individual cases, including epilepsy patients and healthy volunteers?

(2) Does the paradigm used fulfil the diagnostical prerequisite of replicable activation within the amygdala?

(3) Does the use of two distinctive MTL paradigms improve the reliability of MTL diagnostics?

4.3.3 Hypotheses and explorations

In recent neuroimaging studies the amygdala showed the largest and most consistent activation when individuals viewed negative, especially fearful, facial expressions (Zald 2003), even when unattended (Critchley et al 2000; Gorno-Tempini et al 2001; Vuilleumier et al 2001). The comparison of static versus dynamic facial presentations in an fMRI paradigm revealed stronger amygdala activation for dynamic presentations (LaBar et al 2003). Further, the amygdala participates in biological motion such as eye gaze and body movement perception, even when the stimuli have no apparent emotional content (Bonda et al 1996; Kawashima et al 1999).

(1) We hypothesise that the use of an animated fearful face paradigm leads to strong and reliable amygdala-activations in single subjects including epilepsy patients and healthy volunteers.

In recent years, an increasing number of studies using fMRI addresses the activation of the amygdala. The studies mainly addressed group results (Zald 2003) and did not refer to clinical contexts. Up to now, the within-subject reproducibility with regard to MTL structures has not yet been evaluated. From group studies it is known that the amygdala demonstrates rapid habituation (Zald 2003), but this pattern is referred to within-session habituation (Breiter et al 1996; Phillips et al 2001; Thomas et al 2001; Wright et al 2001; Wright et al 2003) and is not transferable to between-session measurements, especially not on a single-case level. However, the use of a paradigm in a clinical context demands that the detected activations are reproducible on an intrasubject level.

(2) We will explore whether the paradigm induces reproducible activations within the amygdala.

To date, fMRI of memory-lateralisation is far from widely replacing the invasive IAT. With recent procedures, the reliability of lateralisation was not higher than around 90% (Killgore et al 1999). Therefore, an increase of the reliability of MTL diagnostics in MTLE patients is highly desirable. We assume, that the use of two distinctive MTL paradigms increases the reliability of MTL diagnostics. This requires that the fearful face task lateralises hemispheric asymmetries in epilepsy patients and that this paradigm reveals additional information by observing dissociations between amygdala and parahippocampal activation.

(3) We will explore whether the use of two distinctive MTL paradigms improves the reliability of MTL diagnostics.

5 Experimental studies

5.1 First study: On the use of caffeine as a contrast booster for BOLD fMRI studies

5.1.1 Introduction

Functional BOLD imaging relies on very small regional changes in the concentration of deoxyhaemoglobin. The mean change in signal intensity in a typical multi-subject fMRI study is ideally somewhere between 1-5%, but in single-case studies, for example in a clinical setting, often even less. Therefore, a considerable amount of research effort has been undertaken to enhance BOLD signal intensity and several pharmacological substrates have been tested for their impact on the BOLD-signal (Li et al 2002). Recently, the administration of methylxanthines such as caffeine or theophylline has been recommended as a BOLD contrast booster for studies confronted with a low SNR (Morton et al 2002; Mulderink et al 2002). The methylxanthines are nonselective adenosine receptor antagonists and block neurovascular receptors, predominantly A2, with a slightly higher affinity than that of neural receptors, predominantly A1 (Dunwiddie et al 2001). Blockade of vascular adenosine receptors produces vasoconstriction and decreases resting cerebral perfusion (Dunwiddie et al 2001). This decrease in baseline cerebral perfusion might enhance the BOLD signal (Dager et al 2000; Mulderink et al 2002), but the relationship between cerebral perfusion and BOLD signal increase following caffeine seems to be complex and not conclusive (Laurienti et al 2003).

In humans, Mulderink (2002) recently reported a considerable overall increase of 26-37% in the BOLD-contrast in a visual (V1) and a motor area (M1) during the performance of a visually cued motor task after administration of 200mg caffeine. In contrast, Laurienti et al. (2002) found that the signal increase in visual cortex induced by visual stimulation following caffeine intake is dependent upon the subjects' dietary caffeine consumption; the magnitude of the BOLD signal was significantly correlated with consumption. Additionally, the BOLD signal change in visual cortex seemed to be restricted to high caffeine users and might be explained by an upregulation of adenosine receptors.

The studies presented above used group-results. However, group results are not necessarily representative for each individual subject. The use of a substance as signal booster should systematically induce a signal increase in each subject. A contrast booster in a clinical context would be helpful because low BOLD contrast is a major problem in the

field of clinical fMRI diagnostics. In particular, the possibility of increased BOLD contrast in the mesial temporal lobe (MTL) would be highly attractive. The MTL is one of the key target regions in clinical fMRI diagnostics, for example in evaluating memory in Alzheimer's disease (Golby et al 2005) or in defining eloquent cortex in the presurgical evaluation of refractory mesial temporal lobe epilepsy (Janszky et al 2005; Richardson et al 2004). Unfortunately, the MTL is difficult to image because of its affinity for susceptibility artefacts induced by its position and the differing magnetic properties of bone, tissue and air in its vicinity (Powell et al 2004).

In addition to the difficulty in obtaining sufficient BOLD contrasts in single-cases, the BOLD signal in patients can also be reduced by pharmacological effects. Antipsychotics, for example, reduced the BOLD signal in the motor systems (Muller et al 2002). Anxiolytics, anticholinergic drugs (lorazepam, scopolamine (Sperling et al 2002)) and antiepileptic drugs (carbamazepine (Jokeit et al 2001b)) have been reported to reduce the BOLD signal within memory-related MTL structures.

The use of a contrast booster would simplify fMRI measurements in clinical contexts and caffeine would be very useful because of its easy and safe administration.

We therefore attempted to reproduce Mulderink et al's group-results of an increased percent BOLD signal change following caffeine in a single case, placebo controlled, repeated fMRI study within three distinct domains, namely, within primary visual and motor area and within memory-related MTL-structures.

5.1.2 Methods

5.1.2.1 Subjects

Six healthy volunteers (five males, aged 24-31) were enrolled in the study. They filled out a questionnaire documenting their level of caffeine use. Four (subjects 1, 3, 4 and 6) were high (2100-2800 mg/week) and two (subjects 2 and 5) low caffeine users (0 and 600 mg/week). Subjects were asked to refrain from ingesting any food or drink containing caffeine (coffee, tea, soft drinks) 12h prior to their imaging time. No subject had been treated with any medication one week prior to the fMRI measurements. Because oestrogen level might have an effect on the size of BOLD-activation (Dietrich et al 2001), cycle phases in the female subject were experimentally controlled over the four measurements: fMRI experiments were conducted between the 12^{th} and 17^{th} day of the menstrual cycle. Subjects gave written informed consent prior to the study after the procedures were fully explained.

5.1.2.2 Experimental design

Subjects performed eight fMRI runs on four different days with intervals of 1-4 weeks. The runs included a visual, a motor and a memory paradigm. Each study day consisted of a pre- and a post-treatment run: Day one: baseline-placebo, day two: baseline - caffeine, day three: baseline - caffeine, day four: baseline-placebo. In the caffeine condition, either 100mg or 200mg of caffeine was administered (counterbalanced: three subjects had the lower dose on the second day, and three subjects had the lower dose on the third day). Post-treatment runs were performed 30 minutes after drug intake.

5.1.2.3 fMRI task design

The visual and the motor task was applied in one blocked paradigm. Seven blocks of the visual paradigm and seven blocks of the motor paradigm were presented in an alternating manner, each block lasted 30 seconds. During the visual block an on-off modulated black-and-white checkerboard was presented at a frequency of 8Hz. In order to keep the subject's attention on the stimulus, red numbers appeared every 3 seconds in the centre of the checkerboard. Subjects were instructed to read this number and to count upwards in steps of two. Finger tapping was employed during the motor block. Subjects were instructed to perform a rhythmic finger-to-thumb movement with their non-dominant hand. Velocity of this finger tapping was 2.6Hz, paced on scanner noise. While tapping, the subjects had to count upwards on steps of two.

The Roland's Hometown Walking Task (Jokeit et al 2001c) was applied to activate memory-related mesial temporal lobe structures. The paradigm consists of ten activation blocks and ten baseline blocks, each with a duration of 30 seconds. During the activation block, the subject has to retrieve spatio-temporal information from long-term memory. For each subject, an individual hometown walk encompassing ten destinations was prepared. Subjects were asked to mentally navigate through the ten different routes and to imagine as many details as possible while navigating. After 30 seconds each route was interrupted by the baseline task. The baseline condition consisted of covertly counting odd numbers starting with 21. In preparation for fMRI, the subject had to fill out a familiarity questionnaire of the landmarks. Only landmarks with high familiarity were included in the individual hometown walks. Blocks with approximate routes (including similar type and similar number of landmarks) of approximately 300 meters were included. To control for memory performance during the fMRI-task, the subject was asked to write down the starting and endpoint of the 10 walks after each fMRI-session. Each subject performed each run adequately as indicated by a sufficient number of recalled landmarks.

5.1.2.4 MR image acquisition

Structural and echo planar functional images were acquired on a 1.5 Tesla Magnetom Sonata Scanner (Siemens, Erlangen, Germany). Subjects were positioned in the head coil with ear pads and foam padding to reduce head motion.

A high resolution T1-weighted anatomical scan was acquired for each subject for reference in single-subject analysis. The parameters for the anatomical sequence were as follows: 176 axial slices with 1mm single-slice thickness, repetition time (TR) 1900ms, echo time (TE) 3.93ms, 15° flip angle, field of view (FOV) 250mm, 256 x 256 matrix.

Functional data were acquired using EPI T2* weighted sequence. The following parameters were applied in the visual, motor and memory paradigm: 20 slices, 5 mm slice thickness (interslice gap: 1mm), repetition time (TR) 3000ms, echo time (TE) 50ms, 90° flip angle, field of view (FOV) 250mm, matrix size 128 x 128 (voxel-size 3.9 x 3.9 x 5mm). In the visual and motor paradigm, axial slices were oriented longitudinal to the AC-PC and in the Roland's Hometown Walking Task, coronal slices were oriented orthogonal to the hippocampal formation covering the whole brain.

5.1.2.5 Data analysis

fMRI data analyses were performed with BrainVoyager 4.9.1 (BrainInnovation, Maastricht, The Netherlands). The data were pre-processed with (1) three-dimensional motion correction and (2) trend removal by temporal fast Fourier transform-based high-pass filtering and transformed into Talairach co-ordinate space (Talairach et al 1988).

For multiple regression analyses, a general linear model (GLM) with the predictor for the activation condition was computed. The time courses of the predictor were obtained by using a linear model of the haemodynamic response. The overall model fit was assessed by using an *F* statistic. Significant differences between the experimental conditions were assessed by using contrast (*t*) maps.

A conjunction analysis was calculated over all subjects to define the region in which every single subject revealed significantly activated voxels. In the centre of the observed activation within the primary motor (M1), and the primary visual (V1) cortices, as well as in the MTL, regions of interest (ROI) were defined by applying a cube with the size of 343 voxels ($1mm^3$ isotropic voxels). Within these ROIs, group analysis were performed using a general linear model (GLM) for the following contrasts: (1) baseline versus caffeine and (2) baseline versus placebo. The contrasts were tested using t-statistics.

For the analysis of the data on single-subject level, we computed in each subject a GLM model including all runs. Then, individual ROIs were defined by applying a cube in the centre of the activation with the size of 343 voxels (1mm^3 isotropic voxels) within the regions previously defined by the conjunction analysis in M1, V1 and MTL. Individual ROIs were used in order to account for anatomical differences among subjects. Within these regions, the data from the ROI were averaged for each individual measurement over the time course to form the average percent BOLD signal change. These data were used for the repeated measurement analysis of variance (RMANOVA). RMANOVA were performed for (1) pre- versus post caffeine administration and (2) pre- versus post placebo administration of averaged percent BOLD signal change in M1, V1 and MTL.

5.1.3 Results

Significant T2* contrast differences were found in each scan within primary motor area (motor stimulation), primary visual area (visual stimulation) and within both MTL (Roland's Hometown Walking Task). In one subject, the first placebo measurement of the Roland hometown walking task had to be excluded due to excessive head motion during the scan. Figure 2 depicts overlap maps of conjunction analyses denoting significant activations in each subject within the target areas.

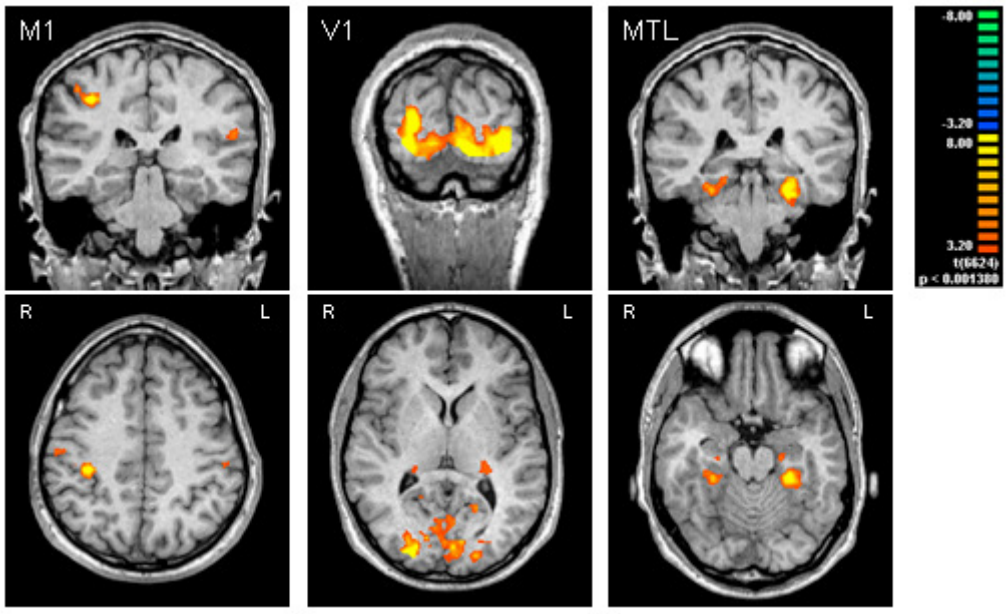

Figure 2: Overlap maps of conjunction analysis denote significantly activated voxels in primary motor area (motor stimulation), visual area (visual stimulation) and mesial temporal lobe (Roland's Hometown Walking Task) in 6 healthy volunteers ($p < .001$).

Following placebo, the average percentage signal change in the BOLD response relative to baseline revealed no difference between baseline and placebo condition in any modality (ROI-GLM in M1: $p = .12$; in V1: $p = .17$ and in MTL: $p = .75$).

Within M1, group analyses revealed a significant signal increase following administration of 200mg caffeine (ROI-GLM: t = 3.25, $p < .001$), whereas the intake of 100mg caffeine did not enhance the average percent BOLD signal (ROI-GLM: t = -2.45, $p = .179$). The significant contrast of 'baseline versus 200mg caffeine condition' is shown in figure 3.

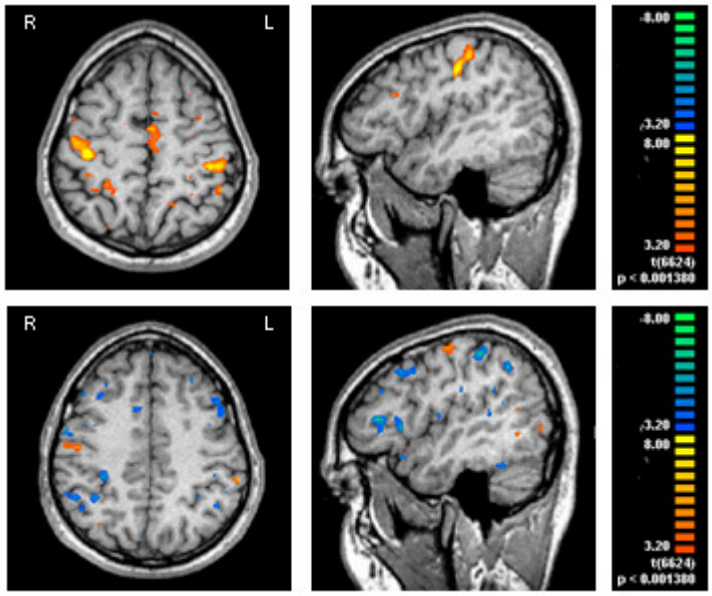

Figure 3: Activation map of the general linear model with the contrasts 'baseline vs. 200mg caffeine' (above) and ‚baseline vs. placebo' (beneath) in the motor task. Note significant increase following 200mg caffeine intake and no significant effect following placebo within primary motor area (M1).

Examination on a single-case level revealed signal increases in five out of six subjects, as indicated in figure 4. Despite that, RMANOVA of the pre- versus post-treatment condition failed to show a significant effect of 200mg caffeine (F (df = 1, n = 6) F = 4.4, $p = .09$, $eta^2 = .47$, Power = .39). Caffeine markedly increased the inter-individual variability (variance coefficient $v = 29\%$) compared to the baseline condition ($v = 17\%$). The percent BOLD signal change after intake of 200mg caffeine positively correlated with average weekly caffeine consumption (r = .80, $p < .05$).

Within V1, average percent BOLD signal change was significantly decreased in group analyses following administration of 100mg (ROI-GLM: t = -4.04, < .00001) and 200mg (ROI-GLM: t = -7.43, $p < .00001$) of caffeine. Figure 5 depicts the significant decrease within V1 of 'baseline versus 200mg caffeine'.

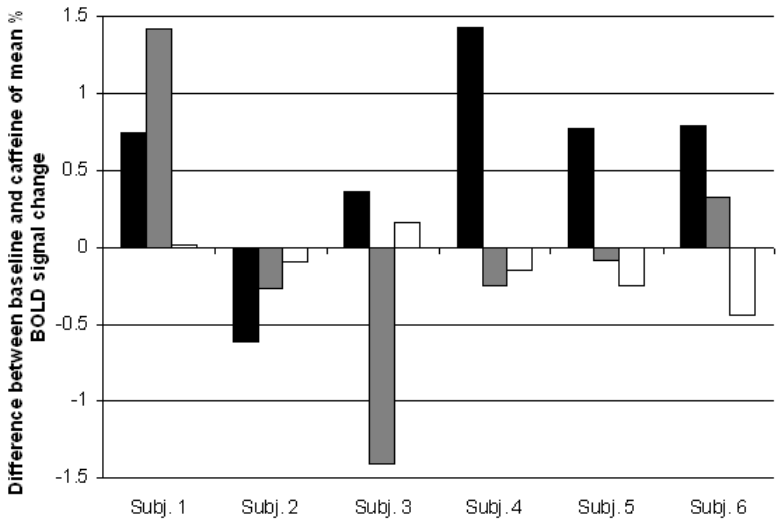

Figure 4: Differences between baseline and caffeine of the mean percent BOLD signal change within the primary motor area (M1, black), the primary visual area (V1, grey) and the mesial temporal lobe (MTL, white).

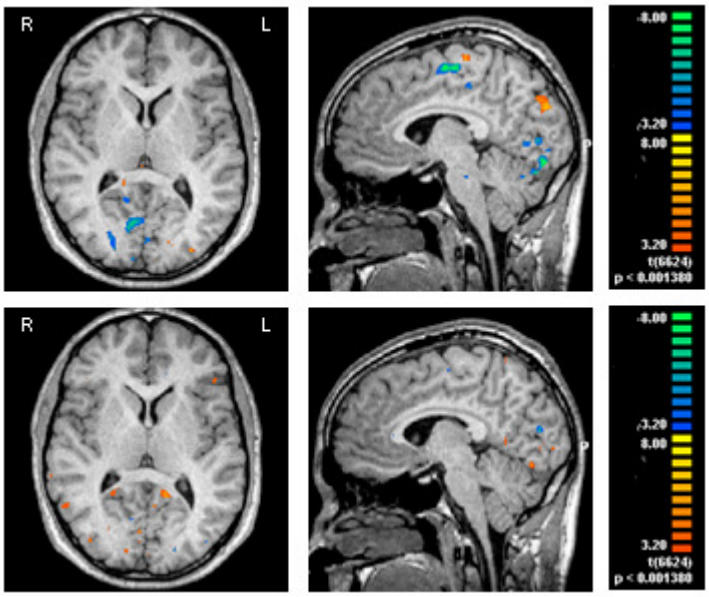

Figure 5: Activation map of the general linear model with the contrasts 'baseline vs. 200mg caffeine' (above) and ‚baseline vs. placebo' (beneath) in the visual task. Note significant decrease following 200mg caffeine intake no significant effect following placebo within primary visual area (V1).

At an individual level we found increases of the BOLD-signal in two subjects and decreases with various magnitude in V1 in four subjects following administration of 200mg caffeine (figure 4). Accordingly, RMANOVA of the pre- versus post-treatment condition

failed to reveal a significant effect of any caffeine dose (100mg caffeine: F (df = 1, n = 6) = .72, p = .44, eta^2 = .13, Power = .11), 200mg caffeine: (F (df = 1, n = 6) = 0.7, p = .81, eta^2 = .02, Power = .06). Within V1, caffeine markedly decreased the inter-individual variability (variance coefficient v = 19%) compared to the baseline condition (v = 37%). Percent BOLD signal change after caffeine intake and average weekly caffeine consumption were unrelated (p = .26).

As the visual and the motor paradigm were applied in one block-design, we looked for a correlation of caffeine-induced signal alterations between these two regions. As indicated in figure 6, we found no systematic correlation between M1 and V1 (spearman's rho = .43, p = .40).

Within MTL no significant alterations following caffeine intake were found, neither for group analyses (ROI-GLM: 100mg caffeine: t = - .86, p = .39 and 200mg caffeine: t = -.05, p = .65), nor in RMANOVA (100mg caffeine (F (df = 1, n = 6) = .004, p = .95, eta^2 = .001, Power = .05) and 200mg caffeine (F (df = 1, n = 6) = .20, p = .20, eta^2 = .30, Power = .22). These group data reflect the results at an individual level: four out of six subjects revealed slightly decreased, one subject slightly increased BOLD signals and one subject did not show any signal alterations following 200mg caffeine (figure 4). Within MTL, caffeine did not have any influence on the inter-individual variability (variance coefficient caffeine: v = 56%, and baseline v = 52%). Again, percent BOLD signal change after caffeine intake and average weekly caffeine consumption were unrelated (p = .82).

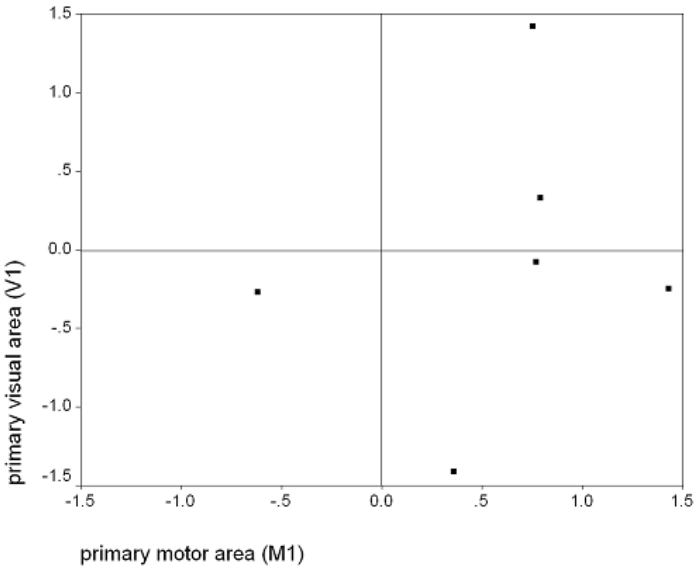

Figure 6: Caffeine-induced signal alterations in primary visual area (V1) compared to primary motor area (M1).

5.1.4 Discussion

Recently, Mulderink and co-workers (2002) suggested the use of caffeine as a BOLD contrast booster for studies confronted with low SNR. The authors reported a considerable overall increase of 26-37% after administration of 200mg caffeine in the BOLD-contrast in a visual (V1) and a motor area (M1) during the performance of a visually cued motor task. We attempted to reproduce the reported group- results by Mulderink and co-workers (2002) in a single case, placebo controlled, repeated fMRI study within primary visual and motor area and adapted these techniques to memory-related MTL-structures.

Our study confirmed the group analyses that 200mg of caffeine enhances the BOLD-contrast in M1 while performing a motor task (ROI-GLM: $p < .001$), although the more conservative RMANOVA showed only a trend ($p = .09$). The increase of BOLD signals following caffeine was clearly correlated with dietary caffeine consumption. This observation is in accordance with Laurienti (2002), who reported significantly higher BOLD signal changes among high caffeine users compared to low caffeine users. However, even if caffeine boosts the percent BOLD signal in group analyses, the results at a single-case level are less convincing. The administration of caffeine led to a marked increase of inter-individual variability. It has therefore to be concluded that the administration of caffeine may boost the BOLD signal in M1 in some subjects, but it can also act as a confounding variable as well.

Within V1 we failed to reproduce the reported signal increase following administration of 200mg caffeine. In contrast, group analyses showed that caffeine decreased the BOLD-signal in V1 ($p < .00001$). Also, at an individual level we found frequent decreases in the BOLD-contrast in V1. The lack of convincing effects was statistically confirmed by applying an RMANOVA model ($p = .81$). This result is in contrast to previous reports of Mulderink et al. (2002) who found a signal increase in V1. However, as can be seen in figure 2a in their report, the standard deviation of the percent BOLD signal change in V1 is about three times higher than the mean, indicating that there are considerable inter-individual differences and deviations from the normal distribution. Therefore, their finding seems to be less convincing than the reports of increased signals following caffeine intake in M1. Our results are in agreement with the results of Laurienti et al. (2002) who demonstrated signal decreases as well as signal increases during a visual paradigm as a function of the individual caffeine consumption. Moreover, they controlled for cerebral perfusion and found that despite consistently reduced perfusion states in all study participants, no more than 47% of the participants demonstrated an increase in BOLD signal intensity in response to caffeine (Laurienti et al 2003).

Within the MTL we failed to find any effects following caffeine administration and moreover, the signal-change was not related to dietary caffeine consumption. Only one subject revealed a slight increase in the BOLD response following caffeine intake.

Regarding the influence of dietary caffeine consumption on the BOLD-contrast, we only found a positive correlation in M1. These results might be in line with the recent observation that the resting cerebral perfusion may be differentially affected in different regions by caffeine (Field et al 2003). The motor cortex belongs to the vascular territory of the anterior and middle cerebral arteries (called the anterior circulation), whereas the primary visual cortex and partially the mesial temporal lobe are supplied by the posterior cerebral arteries (called posterior circulation). The authors found a significant interaction effect between dietary caffeine use and cerebral blood flow (CBF) response to caffeine only in the anterior circulation and not in the posterior circulation. This apparent difference between the studied regions might be explained by regional variations in the number of perivascular adrenergic nerves, adenosine receptors or nucleoside transport proteins (Field et al 2003).

With regard to dosage, the administration of 100mg caffeine did not show any consistent effects on the reported brain areas. The 200mg dose was chosen as Mulderink and co-workers (2002) and Laurienti (2002) administered a caffeine in this range, namely 200mg (Mulderink et al 2002) and 250mg (Laurienti et al 2002).

In summary, our study confirmed with group analyses that 200mg caffeine enhances the BOLD-contrast in M1 while performing a motor task (ROI-GLM: $p < .001$). However, closer examination was less convincing as the use of the more conservative RMANOVA revealed only a trend ($p < .09$), and a marked increase in inter-individual variability was found. Within V1 and MTL, caffeine seems to provide no consistent boosting effects.

On an intra-individual level we found clearly domain-specific effects. Following the administration of caffeine subject one, for example, showed a marked signal increase in M1, whereas caffeine had only a slight influence on V1 and no effect within the MTL. Subject 3 showed (although the signal increased following caffeine intake in M1 and slightly in MTL) a marked decrease in V1. In subject 6 finally, caffeine had a boosting effect in M1 and in V1, whereas the signal decreased in MTL following caffeine. All three subjects were high caffeine users and should, according to the results of Laurienti (2002), show consistent increases in the BOLD signal at least in M1 and in V1.

Although previous studies found some boosting effects on the BOLD response following caffeine administration, we conclude that caffeine seems to be far less effective as a

BOLD contrast booster than expected. Our results discourage the use of caffeine in a clinical context as it may enhance inter-individual variability and may even reduce the signal in certain subjects. Moreover, in our study caffeine related BOLD-contrast changes were clearly domain specific within individual subjects.

5.2 Second study: Reproducibility of serial fMRI scans within MTL

5.2.1 Introduction

The distinct advantages of fMRI include its non-invasiveness, relatively high spatial and for many purposes sufficient temporal resolution, and the ease of imaging underlying anatomy. Serial investigations can be safely performed with fMRI (Turner et al 1997) and therefore it seems to be the method of choice for studying longterm changes in brain function. Possible applications of serial investigations in a clinical setting are the evaluation of functional changes during progressive neurological diseases, especially neurodegenerative disorders such as dementia, the investigation of plastic changes that occur in the brain during recovery, or the examination of the influence of longterm pharmacological treatments such as antiepileptic drugs in epilepsy patients.

The validation of serial fMRI-investigations can be viewed to the retest-reproducibility of the activation pattern (qualitative approach) or to the retest-reproducibility of the activation magnitude and the spatial extent (quantitative approach). The quantitative approach describes any changes within a given structure that occur in the brain during recovery, pathological processes or treatments.

Unfortunately, many factors can affect the BOLD response and may lead to high variability across fMRI sessions. In addition to technical factors such as instability of the scanner or drifts of the mean magnetic field during the scanning session, psychological phenomena (e.g. attention) or physiological factors (e.g. oxygen saturation) can influence the shape, duration and the intensity of the BOLD response. The particular proportion of each of these factors to the variability is still not completely understood. Among the psychological factors, however, it has been shown that the reproducibility of quantitative fMRI results varied with the attentional load showing the highest concordance with high attentional effort (Specht et al 2003). Other observations suggested that global physiological rather than task-related factors contribute to the variability of quantitative fMRI-results: in serial examinations of a visual and a motor paradigm, a high correlation between the variability of the two simultaneously applied paradigms was found (Waldvogel et al 2000). Physiological factors including oxygen saturation (Weckesser et al 1999), diet factors such as lipid ingestion (Noseworthy et al 2003), ingestion of bioactive compounds (Leslie et al

2000) or more complex biological factors such as diurnal factors and phase of menstrual cycle (Dietrich et al 2001) may also cause global activation changes.

Therefore, the evaluation of patients in serial examinations requires validation of the target paradigm with respect to the long-term reproducibility of the results. The longterm-reproducibility of fMRI results with more than three scans has rarely been investigated. Recently, Wei (2004) reported small longitudinal variability of activation in a working-memory task over 9 scans. However, the study used a multi-subject analysis which is not necessarily representative for individual subjects. Investigations in a clinical context require results at a single-subject level which seems to be far more variable. McGonigle and co-workers (2000) repeated a cognitive task (generating random numbers) 33 times on a single subject and found widely varied activated voxels over repeated sessions. High intra-individual intersession variability was also reported in a serial fMRI study with 6 weekly scans in which six subjects performed a visual and a motor task weekly (Waldvogel et al 2000).

To our knowledge, there is only one study reporting quantitative retest-reproducibility of fMRI investigations within the MTL. Using a visual encoding task Machielsen and co-workers (2000) reported substantial variations in the quantitative analysis of fMRI results in three repeated scans on two days. The longterm-reproducibility of repeated fMRI scans is of interest because these structures are among the key target regions in clinical fMRI diagnostics such as the evaluation of memory-related eloquent cortex in Alzheimer's disease (Golby et al 2005) or mesial temporal lobe epilepsy (Golby et al 2005; Janszky et al 2005; Richardson et al 2004).

Therefore, the present study deals with the possibility of investigating longitudinal effects within this region. We used the Roland's Hometown Walking Task (Jokeit et al 2001c) to activate memory-related MTL structures. This paradigm has been shown in many studies to reliably activate the MTL bilaterally on a single-subject level such as in MTLE patients, patients with low formal IQ, children, older subject and subjects from other cultures (Avila et al 2004; Janszky et al 2005; Janszky et al 2004; Jokeit et al 2001b; Jokeit et al 2001c). The advantage of this paradigm is its practicability, reliability and its close relation to every-day memory demands.

We investigated whether this paradigm is robust enough to provide reproducible and detectable activations over a relatively long period. Although we already know that there is a large intra-individual variability in activation across sessions, we aimed to describe the variability over several scans. We explored (1) how stable the data are on a single-subject level and (2) in group analyses. We (3) explored whether there are specific factors such

as behavioural variables or time factors which explain the variability. Answers to these questions are indispensable for distinguishing between the variations inherent in the examination method and the changes in subjects' brain due to recovery, pathological processes or treatment effects.

5.2.2 Materials and methods

5.2.2.1 Subjects

Six healthy volunteers (five males, aged 24-31) were enrolled in the study. The subjects were trained to perform the fMRI tasks which they also rehearsed immediately before undergoing each fMRI scan to minimise any residual learning effects. Because oestrogen level might have an effect on the size of the BOLD-activation (Dietrich et al. 2001), cycle phases in the female subject were experimentally controlled over the four measurements: fMRI experiments were conducted between the 12^{th} and 17^{th} day of the menstrual cycle. All subjects has not been treated with any medication one week prior to the fMRI measurements. Subjects were asked to refrain from ingesting any food or drink containing caffeine (coffee, tea, soft drinks) 12h prior to their imaging time and to refrain from ingesting any food or drink containing ethanol 24h prior to their imaging time. Subjects were instructed to maintain their usual diet. Three subjects were smokers (subject 2, 3, 4). Their smoking habits were assessed and the subjects were asked to maintain time and amount of nicotine intake within the 12h prior to their imaging time. Caffeine, ethanol consumption and smoking habits were reassessed on every scan day. Subjects gave written informed consent prior to the study after the procedure have been fully explained.

5.2.2.2 Experimental design

Subjects were examined six times with a modified version of the Roland's Hometown Walking Task (Jokeit et al 2001c). Measurements were performed on four different days with intervals of 1-4 weeks. Each subject underwent the fMRI measurements at the same time of day to minimise any natural diurnal variations. On the first and the fourth day, two scans were performed. Subjects were repositioned from the scanner between the two scans. On the second and third day, only a single scan was performed (scan number 2 and 3). To summarise, two within-day measurements and four between-day measurements were performed.

5.2.2.3 fMRI task design

The Roland's Hometown Walking Task (Jokeit et al 2001c) was applied to activate memory-related MTL structures. The paradigm consisted of ten activation blocks and ten baseline blocks, each with a duration of 30 seconds. During the activation block, the subject has to retrieve spatio-temporal information from long-term memory. For each subject, an individual hometown walk encompassing ten destinations was prepared. Subjects were asked to mentally navigate through the ten different routes and to imagine as many details as possible while navigating. After 30 seconds each route was interrupted by the baseline task. The baseline condition consisted of covertly counting odd numbers starting with 21. In preparation for fMRI, the subject had to fill out a familiarity questionnaire of the landmarks. Only landmarks with high familiarity were included in the individual hometown walks. Blocks with approximate routes (including similar type and similar number of landmarks) of approximately 300 meters were included. To control for memory performance during the fMRI-task, the subject was asked to write down the starting and endpoint of the 10 walks after each fMRI-session. Each subject performed each run adequately as indicated by a sufficient number of recalled landmarks.

5.2.2.4 MR image acquisition

Structural and echo planar functional images were acquired on a 1.5 Tesla Magnetom Sonata Scanner (Siemens, Erlangen, Germany). Subjects were positioned in the head coil with ear pads and foam padding to reduce head motion.

A high resolution T1-weighted anatomical scan was acquired for each subject for reference in single-subject analysis. The parameters for the anatomical sequence were as follows: 176 axial slices with 1mm single-slice thickness, repetition time (TR) 1900ms, echo time (TE) 3.93ms, 15° flip angle, field of view (FOV) 250mm, 256 x 256 matrix.

Functional data were acquired using an Echo Planar Imaging T2* weighted sequence with the following parameters: 20 slices, 5mm slice thickness (interslice gap: 1mm), repetition time (TR) 3000ms, echo time (TE) 50ms, 90° flip angle, field of view (FOV) 250mm, matrix size 128 x 128 (voxel-size 3.9 x 3.9 x 5mm). Coronal slices were oriented orthogonal to the hippocampal formation covering the whole brain.

5.2.2.5 Data analysis

Functional MRI data analyses were performed with BrainVoyager 4.9.1 (BrainInnovation, Maastricht, The Netherlands). The data were pre-processed with (1) three-dimensional

motion correction and (2) trend removal by temporal fast Fourier transform-based high-pass filtering and transformed into Talairach co-ordinate space (Talairach et al 1988).

For multiple regression analysis, a general linear model (GLM) with the predictor for the activation condition was computed. The time courses of the predictor were obtained by using a linear model of the haemodynamic response. The overall model fit was assessed using an *F* statistic. Significant differences between the experimental conditions were assessed using contrast (*t*) maps.

Number of significantly activated voxels were specified on the functional map in each measurement separately with a predefined statistical threshold of $p < .00001$. The functional cluster was caudally constrained by the crus of the fornix. For each VOI the number of activated voxels was counted in the left and right hemisphere.

To collect the percent BOLD signal change data within the parahippocampal gyrus, VOIs with similar cluster sizes were defined using the following method: A conjunction analysis was computed to define the region in which every run of each subject revealed significantly activated voxels. Then, individual VOIs were defined by applying a cube with the size of 343 voxels ($1mm^3$ isotropic voxels) in the centre of the activation in order to cover the most activated voxels (Bosch 2000). Individual VOIs were used in order to account for anatomical differences among subjects. Within these defined VOIs, time course epochs corresponding to the conditions were averaged together and the percent BOLD signal change was collected for each subject.

We then evaluated the longterm-stability on a single-case level, first by terms of the coefficient of variance (*v)*. It is calculated by dividing the standard deviation by the mean and then by multiplying the result by 100:

$$v = (SD / mean) * 100$$

The advantage of this measure is that it represents a relative standard deviation, since it takes into account the mean. The larger the value, the greater is the variability in the data. In order to describe the longterm-stability intra-individually, we computed the relative deviation from the first scan. A scan was classified to be stable, when the deviation was in between one standard deviation.

To estimate the test-retest reliability by multi-subject analysis, we computed the intraclass correlation coefficient (ICC). This measure was used to quantify the retest-reliability of the activation extent (significantly activated voxels) and the activation intensity (percent BOLD signal change). The ICC represents the difference between within- and between- subject

variability. It approaches 1 when the within-subject variability is low. Most of the observed variance can then be explained by the between-subject variability (Moser et al 1996).

To explore whether the variability depends on time or repetition effects, we computed repeated measurements analysis of variance (RMANOVA).

5.2.3 Results

The modified version of the Roland's Hometown Walking Task led to significant T2* contrast differences within both MTL in all of the 36 measurements ($p < .00001$; except 4th measurement in subj. 6: right MTL $p < .0006$). In one subject, the second scan of the first day had to be excluded due to excessive head motion during the scan.

To quantify the activation, two different dependent variables were exploited in the data analysis. First, we present the spread of activation in terms of significantly activated voxels and second the magnitude of activation, indicated as the percent BOLD signal change.

How stable are the numbers of significantly activated voxels on single-subject level?

Basically the variance coefficients were high in all subjects (table 1). There were subjects with relatively low variance coefficients (e.g. in subject 2) and those with extremely high variance coefficients (e.g. in subject 3).

Table 1: Variance coefficients (*v*) of significantly activated voxels in each subject

Subject	*v* (right MTL)	*v* (left MTL)
1	83%	122%
2	69%	66%
3	115%	142%
4	82%	92%
5	82%	92%
6	75%	88%

There was a high variability of the activation level referred to the number of significantly activated voxels (p < .00001) between subjects (figure 7). Subject one for example activated a relatively low number of significantly activated voxels (mean = 759, ± 578 SD), whereas subject two revealed high numbers of activated voxels (mean = 3305; ± 2012 SD). Within subjects, the numbers of significantly activated voxels varied markedly as indicated in figure 7.

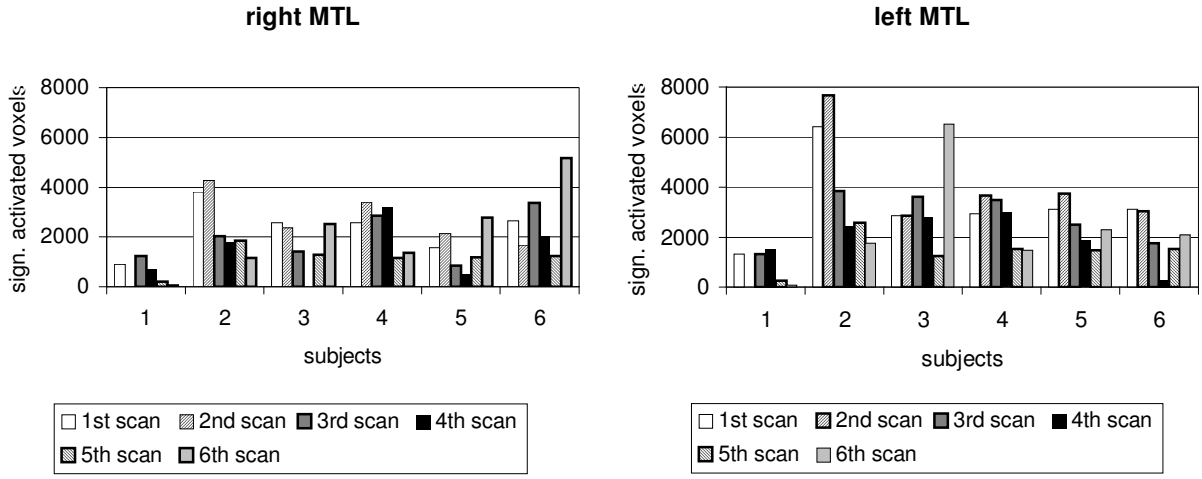

Figure 7: Absolute numbers of significantly activated voxels (p < .00001) in six serial scans in each subject

As far as the level of activation varied between subjects, we investigated their impact on the variances. There was no significant correlation between the mean of significantly activated voxels and variance coefficients (right MTL: spearman's rho = -.72, p = .10, left MTL: spearman's rho = -.23, p = . 66).

In order to describe the longterm-stability intra-individually, we computed the relative deviation of number of significantly activated voxels from the first scan (figure 8). The mean of the relative deviation was within the right MTL 44.7% (± 27.5% SD) and within the left MTL 24.6% (± 34.2% SD). The greatest within-subject variability range was 0.46% to 129% (3, left MTL).

A different approach to describe the stability of scans on a single-subject level is to threshold them as stable, when the deviation from the first scan is less than one standard deviation. Regarding both MTL simultaneously, we found in one single subject (subject 4) three stable scans out of five scan-repetitions (figure 9). Within the scan series and unrelated to the first scan, there were more scans with deviations within one standard deviation (figure 9). All subjects revealed at least two stable scan-repetitions.

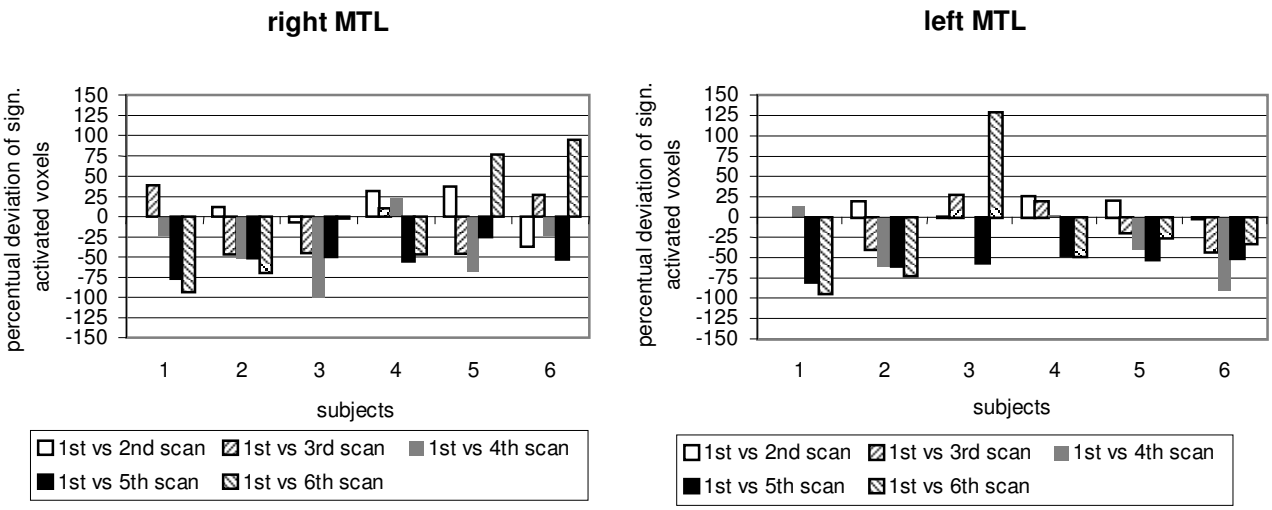

Figure 8: Relative frequency of significantly activated voxels in six serial scans in each subject

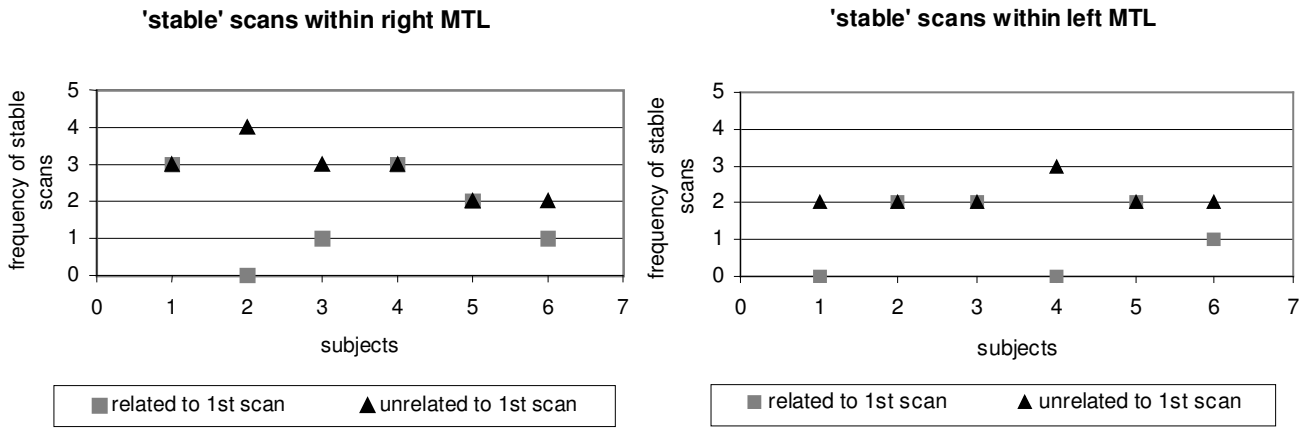

Figure 9: Frequency of 'stable' scans (the deviation from the reference scan was less than one standard deviation) of significantly activated voxels

Are the significantly activated voxels reproducible on group level over scans?

Reproducibility was tested on a group level by performing intraclass correlation coefficients (ICC). The ICC for the significantly activated voxels within the right MTL was .74 and within the left MTL .78. This indicates that the significantly activated voxels were reproducible over scans on group level.

How much vary the numbers of significantly activated voxels on group level?

The mean of the number of significantly activated voxels over all subjects and scans within the right MTL was 1939 (± 1198 SD) and within the left MTL 2628 (± 1655 SD). The variance coefficient within the right MTL was 62% and within the left MTL 63%.

The averaged values over all subjects of the six scans are indicated in figure 10. We computed the relative deviation of averaged number of significantly activated voxels. The reference scan was the first scan. The mean relative deviation between scans of averaged number of significantly activated voxels was 22.4 (± 19.8 SD) in the right MTL and 23.3 (± 22.5 SD) in the left MTL.

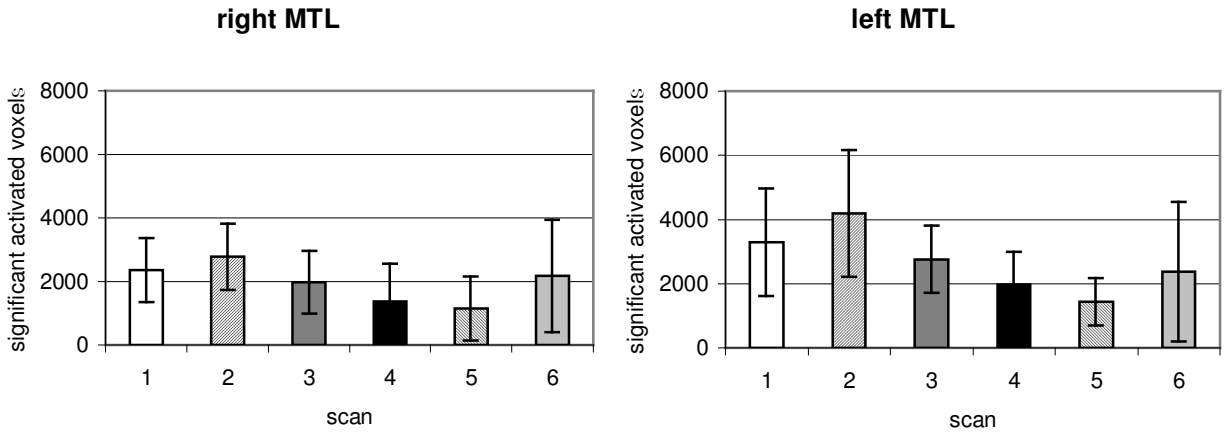

Figure 10: Averages of the significantly activated voxels in six serial scans

Are there specific factors explaining the variability of number of significantly activated voxels?

We investigated whether the following factors might explain the variability of the number of significantly activated voxels:

Days between scans: There was no significant effect whether the scans were performed within one day or between several days: The RMANOVA failed to reveal a significant difference between the scans obtained within one day in contrast to the scans obtained between days of the right- and the left-sided MTL voxel counts (right MTL: $F(1,5) = 1.33$, $p = .31$, $eta^2 = .25$, Power = .15; left MTL: $F(1, 5) = .32$, $p = .15$, $eta^2 = .44$, Power = .28).

Repetition: There was no significant linear decrease over the six scans in the right MTL (RMANOVA: $F(1,5) = .88$, $p = .40$, d = .18, Power = .11) and in the left MTL ($F(1,5) = 2.34$, $p = .20$, d = .37, Power = .22).

Behaviour: Variability was not related to the behavioural performance, as far as no significant correlations were found between the numbers of significantly activated voxels and the numbers of remembered startings and endpoints (right MTL r = .03, p = .88; left MTL r = .06, p = .76).

How stable are the percent BOLD signal changes on single-subject level?

The variance coefficients were in all subjects on a high level (table 2) with extremely high values in subject 5.

Table 2: Variance coefficients (v) of the percent BOLD signal change in each subject

Subject	*v* (right MTL)	*v* (left MTL)
1	114%	83%
2	97%	88%
3	68%	98%
4	72%	83%
5	109%	128%
6	103%	57%

Figure 11 shows the variability of the percent BOLD signal change on a single-subject level. There was a high variability of the activation level referred to the percent BOLD signal change (p < .00001) between subjects (figure 11). The highest percent BOLD signal change was 1.59 (± .69 SD, subject 3), the lowest was .78 (± .30 SD, subject 5).

Activation level had no impact on variability as far as there were no significant correlations between the mean of the percent BOLD signal change and the variance coefficients (right MTL: spearman's rho = -.26, p = .62, left MTL: spearman's rho = .54, p = . 27).

In order to describe the longterm-stability intra-individually, we computed the relative deviation of the percent BOLD signal change from the first scan (figure 12). The mean of the relative deviation was within the right MTL 27.3 (± 17.8 SD) and within the left MTL 27.6 (± 16.8 SD).

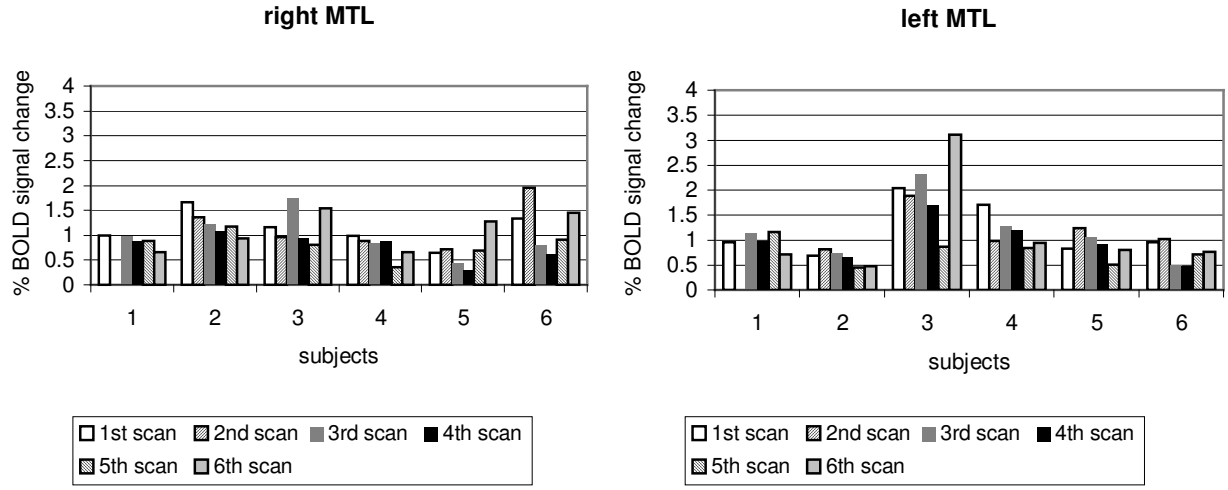

Figure 11: Percent BOLD signal change in six serial scans in each subject

The greatest within-subject variability range was 7.8% to 98.4% (5, right MTL). We considered a repeated scan to be stable when the deviation from the first scan was within one standard deviation. Using this definition, we found no more than three subjects who had two or three 'stable' scan-repetitions (figure 13). Unrelated to the first scan, there were more 'stable' scans. Four out of six subjects (1 to 4) revealed three or four stable scans repetitions within either the left or the right MTL.

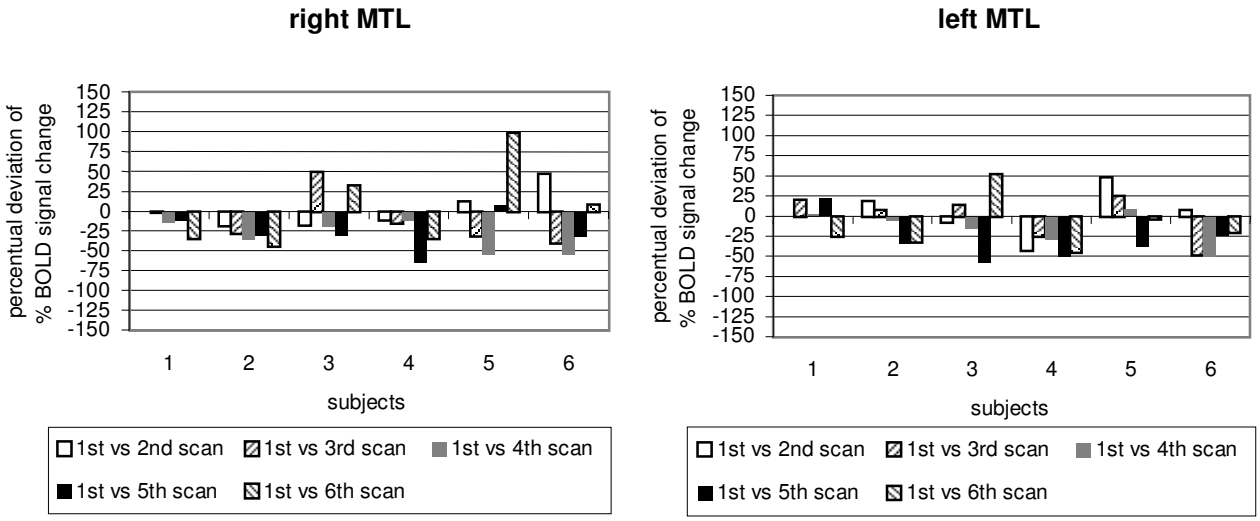

Figure 12: Relative percent BOLD signal change in six serial scans in each subject

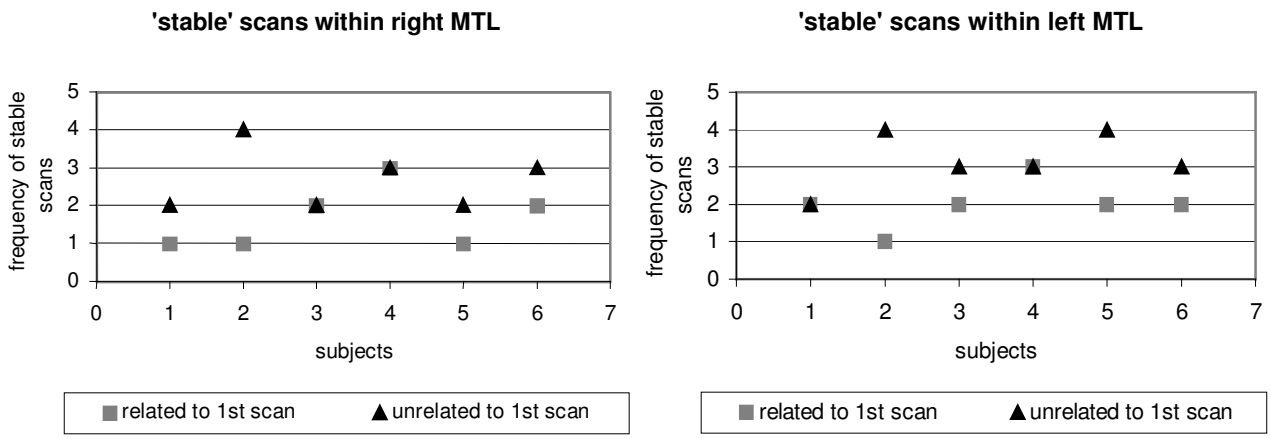

Figure 13: Frequency of 'stable' scans (the deviation from the reference scan was less than one standard deviation) of the percent BOLD signal change

Are the percent BOLD signal changes reproducible on group level over scans?

Reproducibility was tested on a group level by performing intraclass correlation coefficients (ICC). The ICC of the percent BOLD signal change was for the right MTL .73 and for the left MTL .91. This indicates that the significantly activated voxels were on group level reproducible over scans.

How much vary the percent BOLD signal changes on group level?

The mean of the percent BOLD signal change over all subjects and scans within the right MTL was .99 (± .38 SD) and within the left MTL 1.06 (± .57 SD). The variance coefficient within the right MTL was 38% and within the left MTL 54%. The averaged values over all subjects of the six scans are indicated in figure 14.

Are there specific factors explaining the variability of the percent BOLD signal changes?

We investigated, whether the following factors might explain the variability of the percent BOLD signal change.

Days between scans: The RMANOVA failed to reveal a significant difference between the scans obtained within one day in contrast to the scans obtained between days of the percent BOLD signal change within the right MTL and the left MTL (right MTL: $F(1,5) = 1.33$, $p = .31$, $eta^2 = .25$, $p = .15$).

Repetition: There was no significant linear decrease over the six measurements in the right MTL (F(1,5) = 1.02, *p* = .37, d = .20, Power = .12) and in the left MTL (RMANOVA: F(1,5)= 3.6, *p* = .12, d = .48, Power = .31).

Behaviour: The variability was not related to behavioural performance, as there was no significant correlation between the percent BOLD signal change and the numbers of remembered startings and endpoints (right MTL r = -.29, *p* = .16; left MTL r = .18, *p* = .39).

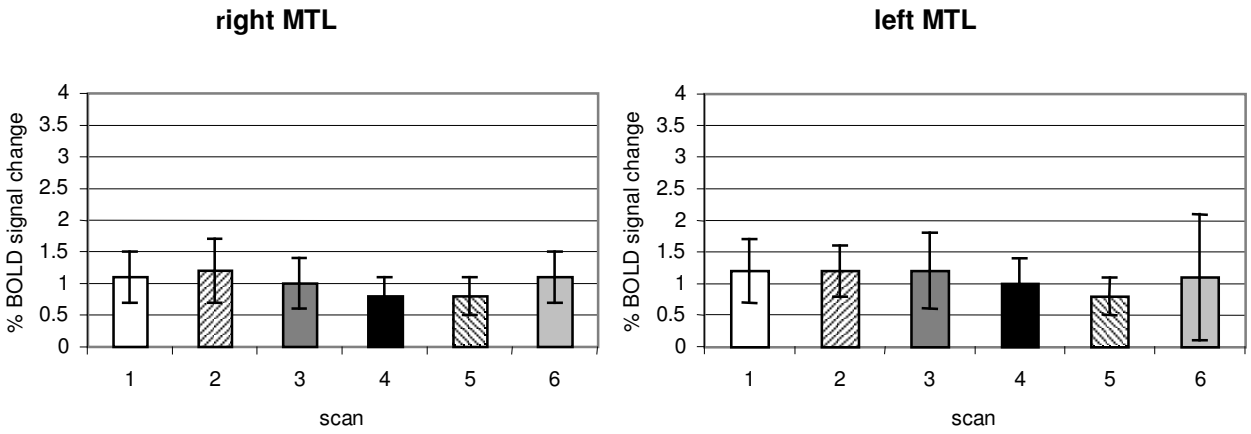

Figure 14: Averages of the percent BOLD signal change in six serial scans

Which measure is more stable: the number of significantly activated voxels or the percent BOLD signal change?

The relative deviations from the first scan were significantly higher in the number of significantly activated voxels compared to the percent BOLD signal change (paired t-test: MTL right: t (23) = 2.7, *p* = .01; paired t-test: MTL left: t (23) = 2.5, *p* = .02). The variance coefficients were comparable in both measure (paired t-test: MTL right: t(5) = -.73, *p* = .49; paired t-test: MTL left: t (5) = .79, *p* = .46). There were no differences between the ICC values of significantly activated voxels compared to the percent BOLD signal change (paired t-test: MTL right: t (14) = -.77, *p* = .45; paired t-test: MTL left: t (14) = -1.9, *p* = .07).

5.2.4 Discussion

The aim of the present study was to describe the longterm reproducibility of fMRI-BOLD signals within the MTL in order to be able in future investigations to distinguish between variations inherent in the examination method and changes in subjects' brain activation

due to functional recovery, pathological processes or treatment effects. As investigations in a clinical context require results on a single-subject level, we first investigated the intra-individual stability of serial fMRI scans.

The Roland's Hometown Walking Task led to significant activations within both MTLs in each scan in each subject. The intra-individual variability in quantitative measures such as the number of significantly activated voxels and the percent BOLD signal change was high across the scans as indicated in several observations. First, in all subjects we observed high variance coefficients. Regarding the number of significantly activated voxels, we found values of up to 142% and regarding the percent BOLD signal of up to 114%. Differences in amount of activation among subjects did not contribute to the level of variability. Subjects with lower amounts of activation (less significantly activated voxels or lower percent BOLD signal change over all six scans) did not show decreased or increased variance coefficients compared to subjects with a higher activation level.

Second, the relative deviations between scans were up to 129% for the number of significantly activated voxels and up to 98% for the percent BOLD signal change. These deviations were in the range of previously observed changes in primary visual and motor areas. The variability of repeated runs ranged intra-individually between zero and several thousand significantly activated voxels (McGonigle et al 2000; Miki et al 2000). Moser and co-workers (Moser et al 1996) reported standard deviations of significantly activated voxels typically in the range of 20-50% but with values of up to 100%. Regarding the percent BOLD signal change Waldvogel and co-workers (2000) observed an extremely high intersession variability in a serial fMRI study in which six subjects performed a visual and a motor task. In the motor task, for example, the percentage of deviation of the activation amplitude from the first scan increased up to 192%.

Third, we found no single subject with consistently stable scans over all repeated scans. A scan was considered to be stable when the relative deviations between scans were within one standard deviation. A higher number of stable scans were observed, when any another scan and not the first scan was the reference. Nevertheless, the most 'stable' subject revealed no more than four stable out of five scan repetitions in either measure. Therefore, one of the main findings of our study is that the variability within our data on a single-subject level was high on both dependent variables, i.e. the number of significantly activated voxels and the percent BOLD signal change. Our study corroborates previous findings of high intra-individual variability of fMRI results observed in primary sensory and motor tasks. The new finding is that we found a high intra-individual variability of fMRI results within the MTL. It has to be concluded that the quantitative interpretation is not a

reliable method to study longterm effects of brain functions at a single-subject level. A quantitative analysis of the data is possibly a more reliable method for the single-case investigation.

The second part of the study investigated quantitative reproducibility on group level. First, we investigated the reproducibility by determining the intraclass correlation coefficient (ICC). The ICC encompasses the difference between within and between-subject variability and approaches one when within-subject variability is low. The ICC values for the number of significantly activated voxels were .74 (right MTL) and .78 (left MTL) and for the percent BOLD signal change .73 (right MTL) and .91 (left MTL). These values indicate a lower contribution of within-subject variability to the overall variability which in turn means, a greater contribution of between-subject variability.

Second, we investigated the amount of variance of the significantly activated voxels and the percent BOLD signal change by calculating variance coefficients. The values were in the midrange, with coefficients between 62% and 63% (right and left MTL) for the number of significantly activated voxels and 38% and 54% (right and left MTL) for the percent BOLD signal change.

A considerable amount of variance may, of course, be explained by global technical (i.e. instability of the scanner or drifts of the mean magnetic field during the scanning session) or physiological factors. We experimentally controlled the physiological factors caffeine, ethanol, nicotine and drug intake over the six scans. Additionally, the scans were performed on the same daytime to rule out any diurnal effects.

We statistically controlled for several factors that might also explain the variability such as time (within-day scans in contrast to between-day scans and repetition effects) as well as behavioural effects. None of these factors explained a significant proportion of the observed variability. Interestingly, neither the comparison of within-day scans to between-day scans, nor repetition effects contributed significantly to the explanation of the variability. Regarding the repetition effects, adaptation or habituation effects might have been expected with increasing time and repetition. Changes related to cognitive practice have also been examined by using fMRI. A common finding in fMRI studies is a reduced haemodynamic response for repeated relative to unrepeated stimuli (Henson et al 2003). However, in contrast to our experiment, previous studies used single session experimental designs. Moreover, the Roland's Hometown Walking Task uses the retrieval from longterm memory traces consolidated several years before the test situation and is therefore probably highly automated.

Machielsen and co-workers (2000) suggested behavioural effects as one possible factor of activation variance. They reported a correlation between behavioural measures (subsequent recognition of pictures which were encoded during scanning) and mean activation volume per subject. In contrast, we observed no behavioural effect explaining the variability by investigating the correlation between the behavioural performance (recalled startings and endpoints) and the numbers of significantly activated voxels or the percent BOLD signal change.

Finally, we found no difference comparing the variance of the number of significantly activated voxels and the percent BOLD signal change. There are controversial suggestions whether to use the number of significantly activated voxels or the amplitude in terms of percent BOLD signal change to define reproducibility. Previous studies observed that the magnitude of activation indicated in percent BOLD signal change is more stable than the number of significantly activated voxels (Cohen et al 1999; Waldvogel et al 2000). On the other hand, Machielsen and co-workers (Machielsen et al 2000) suggested that counting the activated voxels is a reliable method. Of course, the reliability of the selected measure depends on the postprocessing method.

A confounding variable in the number of significantly activated voxel is the statistical threshold chosen. The definition of the threshold is artificial and it might be more appropriate to use individually adjusted thresholds because of the highly variable activation levels between subjects. On the other hand, previous studies showed that different significance levels did not show large deviations in reproducibility when they are near Bonferroni corrected significance thresholds (Rombouts et al 1998). Additionally, the reproducibility of significantly activated voxels can be obtained by using preselected areas. In our study of the MTL, we used a very conservative statistical threshold to count the significantly activated voxels and used predefined regions of interest. A confounding variable with regard to the percent BOLD signal change is probably the selected region of interest, in which the magnitude of activation is defined. Of course, we used a very conservative approach. The region of interest in each subject was predefined by a conjunction analysis and we then applied a small 3D volume in the centre of activation in each scan in order to cover the most activated voxels.

A further main finding of our data is that the quantitative reproducibility on a group level is promising for the investigation of serial fMRI scans. Nevertheless it has to be concluded that there is a considerable amount of variability in the data. Probably one cannot expect a higher stability of cognitive neurophysiological measures in general. Reliability estimates of the P3 component of event-related potentials, for example, were reported to be in the

same range observed in fMRI studies, both over various time intervals (Segalowitz et al 1993; Walhovd et al 2002) and serial examinations (Kinoshita et al 1996).

Because of the remaining substantial variation, investigations of longterm effects on brain functions should rely on group-designs with serial investigations. Additionally, meaningful data can only be expected when strong treatment or recovery effects are anticipated, i.e. with signal changes of certainly more than 30-40%. These estimates are based on the amount of the relative deviation between scans in our study and account for the high standard deviation. Two recent pharmacological fMRI studies probably used potent substances and therefore observed substantial variations in the BOLD response (Jokeit et al 2001b; Sperling et al 2002). Both the anticholinergic drugs lorazepam and scopolamine (Sperling et al 2002) and the antiepileptic drug carbamazepine (Jokeit et al 2001b) considerably decreased the BOLD signal within the MTL. Compared to a placebo-condition, lorazepam and scopolamine decreased the significantly activated voxels within the hippocampus by approximately 40-60% and the percent BOLD signal change by approximately 50-60%. When only high doses of carbamazepine (serum levels of more than 7 mg/l) are included, the proportion of significantly activated voxels within the MTL was decreased by approximately 50% as compared to a control group. As the latter study used a cross-sectional study design, it is necessary to replicate this result in a longitudinal group-study.

To summarise, we found substantial intra-individual variability in both the number of significantly activated voxels and the percent BOLD signal change within the MTL in six serial fMRI scans. We concluded that the quantitative interpretation is not a reliable method for studying longterm effects of brain functions at a single-subject level. However, at a group level, we found reasonable reproducibility, indicated by sufficient ICC values. These results should not discourage the application of serial fMRI scans to investigate the plasticity of changes, to evaluate the functional evolution of chronic neurological diseases, or to study the influence of longterm pharmacological treatments. However, its application is limited to its use in longitudinal group studies and to the evaluation of substantial influences on the brain functions such as pharmacological agents with a high impact.

5.3 Third study: Amygdala fMRI in mesial temporal lobe epilepsy: feasibility, reproducibility, and clinical experiences

5.3.1 Introduction

Mesial temporal lobe epilepsy (MTLE) is the most frequent form of focal epilepsy in adults (Engel et al 1997), and epileptic seizures typically arise from the inner parts of the MTL (Mathern et al 2002). Mesial temporal lobe epilepsy is typically characterised by sclerosis of the hippocampal formation (Blumcke et al 2002). Hippocampal sclerosis is most often found in conjunction with amygdala damage, but, as indicated by analysis of histological sections and MRI, may also be restricted solely to the amygdala (Blumcke et al 2002; Miller et al 1994; Pitkanen et al 1998). In the majority of patients, MTLE is refractory to medical treatment (Kim 2001). In these patients, temporal lobe surgery is the treatment of choice. Standard surgical techniques are selective amygdala-hippocampectomy and anterior temporal lobectomy with resection of the hippocampal formation, the amygdala, and, depending on the surgical approach, lateral temporal neocortex (Kim et al 2001).

The high vulnerability and epileptogenic disposition of the hippocampus and adjacent anatomically related MTL structures coincides with its importance for learning and memory (Eichenbaum 1999; McGaugh 2000). Unilateral MTL damage typically leads to material specific memory impairment (Hermann et al 1992; Pillon et al 1999). Thus, memory deficits are a major complication of TLE surgery (Kapur et al 2003; Pilcher et al 1993).

Beyond the function of MTL structures in memory, the anterior temporal lobe (TL), namely the amygdala, is one of the key structures involved in emotional processing. Lesion and imaging studies emphasise its importance in conditioning of autonomic responses (Bechara et al 1995), emotional memory (Brierley 2004; Cahill et al 1995), and in social perceptual skills such as recognising facial expression (Adolphs 2003), and emotional behaviour (Tranel et al 1990). Impairments following unilateral TL surgery may thus cause problems in emotional processing, including social behaviour and specific functions of social and emotional cognition (Adolphs et al 2001; Anderson et al 2000; Brierley et al 2004; Hermann 2002). However, the question of whether certain impairments following TL surgery are related to resected parenchyma of the amygdala is a matter of controversy.

Due to the functional importance of MTL structures, the benefit of surgery must be assessed relative to possible deficits. Presurgical diagnostics aims at identifying eloquent cortex to avoid disabling deficits. To date, presurgical diagnostics in refractory MTLE patients has mainly addressed memory-related structures. In contrast, postsurgical changes related to different degrees of amygdalar dysfunctions have not been widely studied. However, amygdala-related functions are receiving increasing attention in neuropsychological diagnostics (Benuzzi et al 2004; Glogau et al 2004; Meletti et al 2003).

The intracarotid amobarbital test (IAT) is currently the most common and reliable method used to assess lateralisation of memory (Loring et al 2001b). The IAT anaesthetises an entire hemisphere in order to test the other hemisphere for verbal and visual encoding or retrieval efficiency. However, this invasive procedure is difficult to repeat and associated with rare but significant complications. Standard protocols of invasive IAT do not provide in any patient reliable information because of limited time and because the injected barbiturate may not always anaesthetise the area of interest. With the use of presurgical fMRI diagnostics it is hoped that this new technique might supersede the invasive IAT (Jokeit et al 2001a; Richardson et al 2004). Apart from the fact that fMRI is non-invasive, it provides the possibility of mapping additional structures such as the amygdala.

Today, fMRI is an attractive tool for examining memory-functions in presurgical diagnostics due to its wide availability, minimal risk and low costs. Functional MRI paradigms normally activate the perihippocampal regions bilaterally. Accordingly, the failure to activate the left or the right MTL indicates impaired function (Vingerhoets et al 2004). A few groups have begun to asses the utility of fMRI for lateralising memory function in presurgical testing of patients with medically refractory TLE using episodic memory encoding paradigms with complex visual scenes (Detre et al 1998; Stern et al 1996) or multimodal stimuli (Golby et al 2002; Kelley et al 1998). Jokeit and co-workers (2001c) have shown that a simple fMRI memory paradigm (Roland's Home Town Walk) effectively lateralises the epileptic temporal lobe by activation of the parahippocampal gyri. The main technical problem of fMRI within the temporal lobes is its susceptibility to artefacts caused by inhomogenities in the field due to the differing magnetic properties of bone, tissue and air (Powell and Koepp, 2004). The temporal lobe, with its different tissues, is therefore especially likely to suffer from geometric distortions or loss of BOLD signal (Jezzard et al 1999). The latter artefact is serious as signal loss leads to sensitivity loss. It has been shown that this artefact is most prominent in the inferior frontal and inferolateral temporal region (Ojemann et al 1997). Susceptibility-induced signal loss is greater in the anterior relative to posterior hippocampus. This may be the reason for the

relative lack of anterior hippocampal activation in fMRI studies of memory (Greicius et al 2003). In spite of these difficulties, amygdala imaging might help to boost clinical interest in amygdala-related functions, as well as improving presurgical diagnostics of MTL for lateralisation reliability.

Due to its small volume and position in the anterior part of the temporal lobe, the amygdala is difficult to image with fMRI. Furthermore, the use of a paradigm in a clinical context requires that the paradigm activates the amygdala sufficiently and provides reliable, reproducible activations in each individual patient, regardless of their cognitive status. In recent neuroimaging studies the amygdala showed the largest and most consistent activation when individuals viewed negative, especially fearful, facial expressions (Zald 2003), even when unattended (Critchley et al 2000; Gorno-Tempini et al 2001; Vuilleumier et al 2001). Comparison of static versus dynamic facial presentations in an fMRI paradigm revealed stronger amygdala activation for dynamic presentations (LaBar et al 2003). This highlights the importance of temporal cues in the neural coding of facial expressions. Further, the amygdala participates in biological motion perception such as eye gaze and body movement perception, even when the stimuli have no apparent emotional content (Bonda et al 1996; Kawashima et al 1999).

Based on these insights, we developed an fMRI paradigm using visual presentation of sequences from thriller and horror movies showing actors portraying fearful faces. This paradigm was aimed at improving presurgical diagnostics in two ways: (1) The use of two distinctive fMRI-paradigms mapping both the amygdala and the parahippocampal region might improve the reliability of MTL diagnostics in MTLE patients. (2) In future, presurgical imaging of the amygdala may allow for the evaluation of possible clinical implications of amygdala-resections and may thus help to identify candidates at risk of emotional and social impairment following temporal lobe surgery.

Therefore, the first aim of our study was to investigate the feasibility of imaging the amygdala in individual cases, including epilepsy patients and healthy volunteers. Second, we evaluated in healthy volunteers whether the paradigm fulfils prerequisites of clinical diagnostics such as bilateral, strong, reliable and reproducible activations. And third, we asked whether the use of two paradigms that activate MTL structures, the fearful face task and the Roland's Hometown Walking Task, improves the reliability of MTL diagnostics. This would require that the fearful face task lateralises hemispheric asymmetries in epilepsy patients and that this paradigm reveals additional information by observing dissociations between amygdala and parahippocampal activation.

5.3.2 Materials and methods

5.3.2.1 Subjects

Seventeen patients (11 male, 6 female) aged 18-58 years with symptomatic focal epilepsy were investigated (table 3). Twelve of these patients suffered from MTLE (11 hippocampal sclerosis, one neoplasia) with clear unilateral seizure onset of temporal origin (6 right, 6 left) as shown by continuous interictal and ictal video/EEG monitoring with scalp and sphenoidal electrodes. Five patients had lesions outside the MTL. Lesions and structural integrity of non-affected MTL were demonstrated by high-resolution routine MRI. Language laterality in all patients was defined using an fMRI-paradigm employing verbal fluency (Jokeit et al 2001b). Eleven epilepsy patients had left-sided, two bilateral and one had right-sided language dominance (in three patients language dominance was not available due to excessive head motion during the scan).

Seventeen control subjects (aged 23 to 57 years, mean = 31 (± 7.4 SD) 8 female, 14 right-handed) were included. Fourteen were self-reported right-handed and three left-handed. All were healthy with no history of psychiatric or neurological illness and were free of medication.

In all subjects, including the 17 epilepsy patients and 17 healthy volunteers, amygdala activation was measured with a fearful face paradigm. In order to test intrasubject reproducibility, 12 healthy volunteers (5 females, 4 right-handed) were rescanned one to eight weeks later.

In addition to the fearful face paradigm, parahippocampal activation was measured in the same session in all patients and in nine of the control subjects (5 female, aged 24-38) using the Roland's Home Town Walking Task (Jokeit et al 2001c).

After a complete explanation of the study, all subjects gave written informed consent. The investigations performed here were in compliance with the code of ethics as stated in the Declaration of Helsinki and were approved by the local Medical Ethics Committee.

5.3.2.2 fMRI task design

In order to activate the amygdala, we developed a paradigm utilising visual presentations of dynamic fearful faces. Stimuli were presented in a block-design. The paradigm consisted of eight activation and eight baseline blocks each lasting 24 seconds. The activation condition consisted of 75 brief episodes (2-3 sec) from thriller and horror films.

All episodes showed the faces of actors who were expressing fear with high-intensity. None of the episodes showed violence or aggression.

Quality and applicability of film sequences were evaluated by an expert panel consisting of nine psychologists. Out of an initial collection of 120 scenes, only sequences that were considered appropriate by the majority of the expert panel were extracted for the paradigm. Evaluation criteria were as follows: (1) actor's face is clearly visible (2) emotion displayed is clearly recognisable as fear (3) fear is the only clearly recognisable emotion, no other emotion (e.g. anger, sadness, surprise) is displayed, and (4) the fear displayed is of high intensity.

During baseline blocks, 72 short episodes of similar length (2-3 sec) with dynamic landscape video recordings were presented. Video clips of dull domestic landscapes were used due to their stable low emotional content while general visual stimulus properties were comparable to the movie clips. Frequency and duration of the sequences (2-3 sec) were matched for activation and control conditions. Stimuli were presented via a back-projection screen, viewed through a tilted overhead mirror. Prior to beginning, subjects were told that they would see rapid presentations of film sequences depicting fearful faces intermixed with landscape film sequences. They were instructed to relax while watching the film and to focus on the eyes of the actors during the activation blocks. After the scan, subjects were asked to rate the emotional involvement they felt on a continuous scale from zero to ten. Furthermore, subjects were asked to indicate which movies they recognised.

Roland's Hometown Walking Task (Woermann et al 2003) was used to induce memory-related activation within the MTL. The paradigm consisted of ten activation blocks and ten baseline blocks, each with a duration of 30 seconds. During the activation blocks, subjects were required to retrieve spatio-temporal information from long-term memory. For each subject, an individual hometown walk encompassing ten destinations was prepared. Subjects were asked to mentally navigate through the ten different routes and to imagine as many details as possible while navigating. After 30 seconds each route was interrupted by the baseline task. The baseline condition consisted of silently counting odd numbers starting with 21.

We used a verbal fluency task (Woermann et al 2003) to determine language dominance. In each patient, 50 sets of images sampled during five episodes of silent word generation were contrasted with 50 sets of images from 5 episodes of resting inactivity (patients were instructed to stop producing words) using a block design.

5.3.2.3 MR image acquisition

Structural and echo planar imaging functional images were acquired on a 1.5 Tesla Magnetom Sonata Scanner (Siemens, Erlangen, Germany). Subjects were positioned in the head coil with ear pads and foam padding to reduce head motion.

A high resolution T1-weighted anatomical scan was acquired for each subject for reference in single-subject analysis. The parameters for the anatomical sequence were as follows: 176 axial slices with 1mm single-slice thickness, repetition time (TR) 1900ms, echo time (TE) 3.93ms, 15° flip angle, field of view (FOV) 250mm, 256 x 256 matrix.

Functional data were acquired using EPI T2* weighted sequence. The following parameters were applied to measure amygdala activation: 12 coronal slices, 5mm slice thickness (interslice gap: 1mm), repetition time (TR) 1490ms, echo time (TE) 60ms, 90° flip angle, field of view (FOV) 250mm, matrix size 64 x 64 (voxel-size 3.9 x 3.9 x 5mm), reconstructed into an image matrix of 128 x 128. Coronal slices were oriented orthogonal to the hippocampal formation and covered the anterior TL.

In Roland's Hometown Walking Task and the word generation paradigm the following parameters were applied: 21 slices, 5mm slice thickness (interslice gap: 1.2mm), repetition time (TR) 3000ms, echo time (TE) 50ms, 90° flip angle, field of view (FOV) 250mm, size 64 x 64 (voxel-size 3.9 x 3.9 x 5mm), reconstructed into an image matrix of 128 x 128. In Roland's Hometown Walking Task coronal slices were oriented orthogonal to the hippocampal formation and in the word generation paradigm, axial slices were oriented longitudinal to the AC-PC.

5.3.2.4 Data analysis

fMRI data analyses were performed with BrainVoyager 4.9.1 (BrainInnovation, Maastricht, The Netherlands). Prior to analysis, the data were pre-processed with (1) three-dimensional motion correction and (2) trend removal by temporal fast Fourier transform-based high-pass filtering and transformed into Talairach co-ordinate space (Talairach et al 1988). Images of the fearful face task were additionally spatially smoothed with a full width at half maximum of 4mm.

For multiple regression analysis, a general linear model (GLM) with the predictor for the activation condition was computed. The time courses of the predictor were obtained by using a linear model of the haemodynamic response. The overall model fit was assessed using an *F* statistic. Significant differences between the experimental conditions were assessed using contrast (*t*) maps.

Language lateralisation was determined visually by evaluation of frontolateral and temporoposterior lateral as well as parietal and frontomesial activation for asymmetry with individual statistical thresholds (Woermann et al., 2003).

For images in the fearful face task, individual volumes of interests (VOIs) were defined for the amygdalar region. VOIs were specified functionally in each patient separately on a predefined statistical threshold of $p < .001$ ($z > 3$). This threshold accounted for the small size of the periamygdalar region. The size of the periamygdalar region was between 60 and 100 voxels (3.9 x 3.9 x 5mm). A threshold of $z > 3$ ensured that the error probability for a single activated voxel within the region of interest is smaller than 0.05 (one-tailed test). Anatomical border of the functional clusters was the uncal recess caudally and the optical chiasm rostrally and white matter superiorly. For each VOI, the number of activated voxels was counted in the left and right hemisphere.

The same method was used for parahippocampal VOIs in Roland's Hometown Walking Task. The predefined statistical threshold was defined as $p < .00001$ (Jokeit et al., 2001c) and the functional cluster was caudally constrained by the crus of the fornix.

In epilepsy patients and healthy volunteers, lateralisation indices (LI) were defined for number of significantly activated voxels within parahippocampal gyrus and amygdalar/ periamygdalar region using the formula:

$$LI = (right-left) / (right + left)$$

The categorisation of memory lateralisation was defined based upon the results of the healthy volunteers. The mean of LI in healthy volunteers was .05 (± .18 SD). If the LI was greater than 0.23 or less than -.13 we categorised these cases as lateralised memory representation. The mean LI of the amygdala was .23 (± .24 SD). If the LI was greater than .47 or less than -0.01 we categorised these cases as lateralised amygdala representation.

For healthy volunteers, data from the fearful face task were evaluated with respect to reliability, strength and reproducibility of activation. Reliability of activation was investigated with a conjunction analysis over all 17 subjects. To collect percent BOLD signal change data within the amygdala, VOIs with similar cluster sizes were defined using the following method: A conjunction analysis was computed to define the region in which every single subject revealed significantly activated voxels. Then, individual VOIs were defined by applying a cube with the size of 125 voxels ($1mm^3$ isotropic voxels) in the centre of the activation in order to cover the most activated voxels . Individual VOIs were used in order to account for anatomical differences among subjects. Within these defined

VOIs, time course epochs corresponding to the conditions were averaged together and peak of percent BOLD signal change was collected for each subject.

To analyse reproducibility of the amygdala-paradigm, we looked for between-session habituation effects and different test-retest reliability estimates. Between-session habituation was analysed using RMANOVA with the factors 'significantly activated voxels' and 'percent BOLD signal change'. Additionally, ROI GLM analysis with separate study predictors were performed. The ROI based time courses were then statistically evaluated for the contrast first measurement versus second measurement. This contrast was tested using t-statistics.

To estimate test-retest reliability we computed intraclass correlation coefficient (ICC) and percentage overlap ($R^{ij}_{overlap}$). ICC was used to quantify the reliability of activation extent (significantly activated voxels) and activation intensity (percent BOLD signal change). ICC represents the difference between within- and between-subject variability and it approaches 1 when within-subject variability is low. Most of the observed variance can then be explained by between-subject variability (Specht et al 2003). Rij overlap was used to investigate test-retest reliability of activation location. $R^{ij}_{overlap}$ between two activation maps was calculated as follows (Rombouts et al 1998):

$$R^{ij}_{overlap} = 2*V_{overlap} / V_i + V_j$$

$V_{overlap}$ was the volume activated in both the first and second measurement. V_i indicates the number of activated voxels in the first measurement, V_j in the second measurement.

5.3.3 Results

In all subjects, including the 17 epilepsy patients and 17 healthy volunteers, significant T2* contrast differences were found within the amygdala ($p < .001$) in response to watching fearful faces from horror and thriller movies in contrast to watching landscape scenes. The activation focus was located in the superior part of the amygdala (Talairach co-ordinates: 20 –4 –8 (right) and –20 –5 –7 (left)).

Characteristics regarding the clinical usefulness of the fearful face paradigm were first evaluated in healthy volunteers. The fearful face task led to significant bilateral activation in every control subject as demonstrated by significant voxels in this region following conjunction analysis ($p < .05$, figure 15).

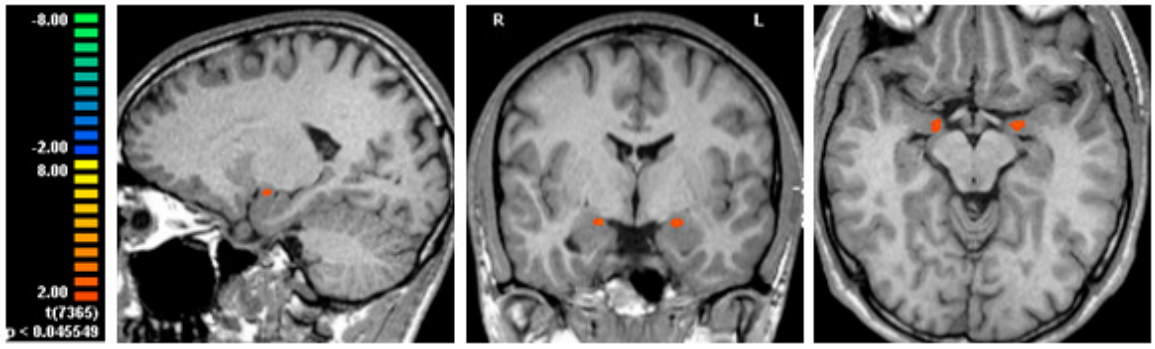

Figure 15: Conjunction analysis of the amygdala-activations induced by the fearful face task in healthy volunteers. Overlap maps denote bilateral significantly activated voxels within the amygdala in all 17 healthy volunteers ($p < .05$).

The mean percent signal change of the BOLD response relative to baseline was 1.09% (± .30 SD) in the right and .81% (± .28 SD) in the left amygdala. Significant changes of BOLD response in single subject exceeded 0.67% (± .20% SD) within the right amygdala and 0.51% (± 0.22% SD) within the left amygdala. Activation intensity was not correlated with self reported strength of emotional involvement ($p > .33$) or recognition of movie sequences ($p > .60$), whereas recognition of movies and felt emotional involvement was negatively correlated (r= -.52, $p < .05$).

Bilateral amygdala activations were reproducible in all 12 subjects. The number of significantly activated voxels and percent BOLD signal change for both measurements were comparable for all twelve subjects (figures 16 and 17). There was no between-session change in the number of significantly activated voxels (RMANOVA right MTL: F (df = 1, n = 12) = 2.87, $p = .12$, $eta^2 = .20$, Power = .34 and RMANOVA left MTL: (F (df = 1, n = 6) = .005, $p = .95$, $eta^2 = 0$, Power = .05) or percent BOLD signal change (RMANOVA right MTL: F (df = 1, n = 12) = .17, $p = .32$, $eta^2 = .13$, Power = .22, RMANOVA left MTL: F (df = 1, n = 12) = .53, $p = .51$, $eta^2 = .11$, Power = .09). In addition, the ROI GLM analysis revealed no significant differences for the contrast 'first measurement versus second measurement' over all subjects (left amygdala: $p = .98$; right amygdala: $p = .42$). Retest-reliability, as measured by ICC, was high for significantly activated voxels (right: ICC = .92, $p < .0001$; left: ICC = .79, $p < .005$) and percent BOLD signal change (right: ICC = .69, $p < .05$; left: ICC = .83, $p < .005$). The relative amount of overlapping volumes demonstrates that the measurements constantly activated voxels in the amygdala which were significantly activated during both the first and the second measurement. Depending on the threshold chosen, slightly different percentages of Rij overlap were found. Mean overlap with $p < .001 = .36$ (± .19 SD), with $p < .005 = .39$ (± .18 SD), and with $p < .01 = .46$ (± .19 SD).

Table 3: Demographic and clinical data for the patients studied

Pat.	Type of epilepsy	Side of seizure onset	Morphologic lesion	Age at seizure onset (y)	Duration of epilepsy (y)	Language	Amygdala activation	LI (p<.001)	Parahipp. activation	LI (p<.00001)
A1	MTLE (th. r.)	right	HS	4	37	left	bilateral	.08	left > right	-.61
A2	MTLE	right	HS	0	25	n.a.	unilat. left	-1	unilat. left	-1
A3	MTLE (th. r.)	right	HS	7	11	left	left > right	-.78	left > right	-.79
A4	MTLE	right	HS	27	3	left	left > right	-.28	left > right	-.48
A5	MTLE (th. r.)	right	Amygdala lesion	18	3	left	unilat. left	-1	left > right	-.92
A6	MTLE (th. r.)	right	HS	11	45	bilateral	unilat. left	-1	right > left	.32
B1	MTLE (th. r.)	left	HS	1	30	left	unilat. right	1	right > left	.48
B2	MTLE (th. r.)	left	Tumour	37	8	left	unilat. right	1	Left > right	-.23
B3	MTLE	left	HS	7	26	left	unilat. right	.96	right > left	.77
B4	MTLE	left	HS	4	43	left	unilat. right	1	right > left	.40
B5	MTLE (th. r.)	left	HS	9	35	left	unilat. right	1	right > left	.44
B6	MTLE	left	HS	13	20	left	unilat. right	.8	right > left	.52
C1	TLE	left	Perinatal damage within left sylvian fissure	19	1.5	right	bilateral[1]	-.35	bilateral	.23
D1	FLE	left	Cortical atrophy frontal/dorsoparietal	15	5	n.a.	bilateral	.02	bilateral	-.01
D2	n.a.	n.a.	n.a. (TL not affected)	22	16	left	bilateral	.15	bilateral	0
D3	PLE	left	Meningeoma parieto-occipital	44	1	n.a.	bilateral	.34	bilateral	.05
D4	PLE	left	Resected Oligo-dendroglioma within left paracentral gyr.	39	.5	bilateral	bilateral[1]	-.25	bilateral	.22

n.a. not available; MTLE (th. r.) = Mesial temporal lobe epilepsy (Therapy refractory); TLE = Temporal lobe epilepsy; PLE = Parietal lobe epilepsy; FLE = Frontal lobe epilepsy; HS = Hippocampal sclerosis; LI = Lateralisation index; [1]reversed LI is presumed due to atypical language dominance

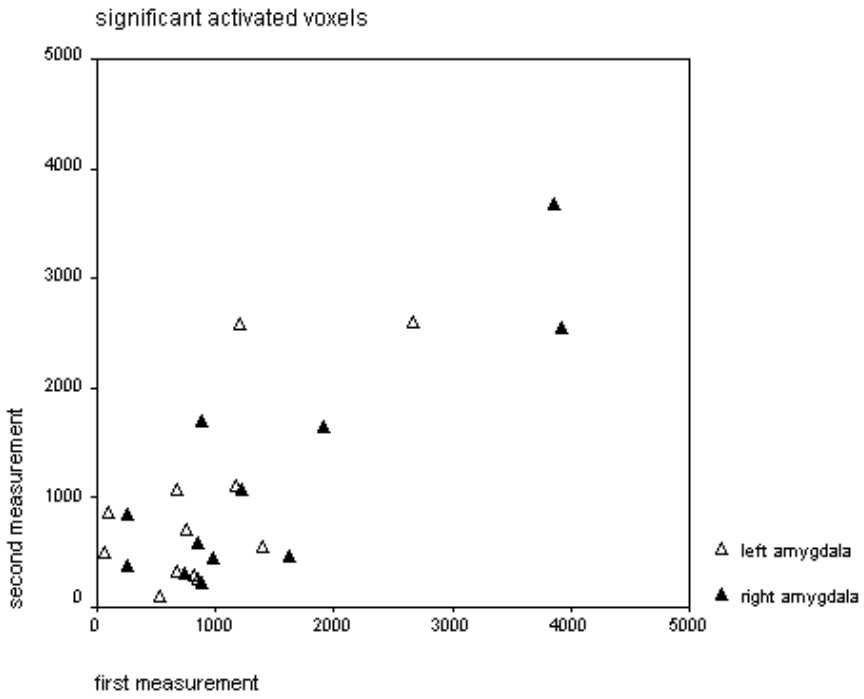

Figure 16: Scatterplot of significantly activated voxels induced by the fearful face task during the first and the second measurement. Note the high reproducibility of the significantly activated voxels within the amygdala.

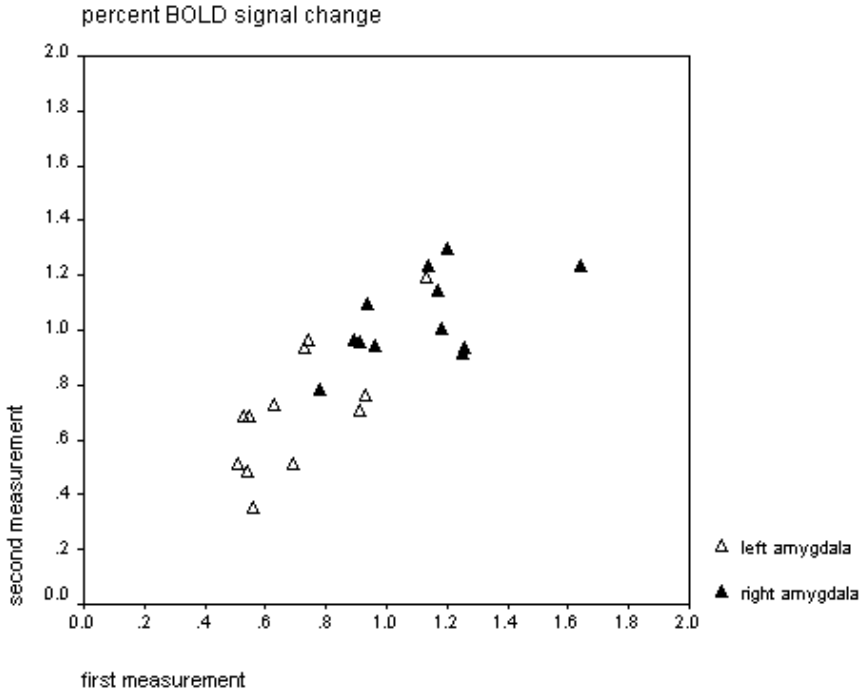

Figure 17: Scatterplot of percent BOLD signal change induced by the fearful face task during the first and the second measurement. Note the high reproducibility of the percent BOLD signal change within the amygdala.

The clinical usefulness of the fearful face paradigm was further analysed with regard to the explanatory power of lateralisation in epilepsy patients as compared to controls. The bilateral amygdala-activation in control subjects (mean of LI = .23 (±. 24 SD)) and patients without MTLE was slightly asymmetric to the right side, but did not significantly differ from zero. In contrast, eleven of the 12 patients with MTLE showed clearly lateralised amygdala activation contralateral to the side of seizure onset (figure 18). Seven of these patients had even unilateral amygdala activation (Table 3).

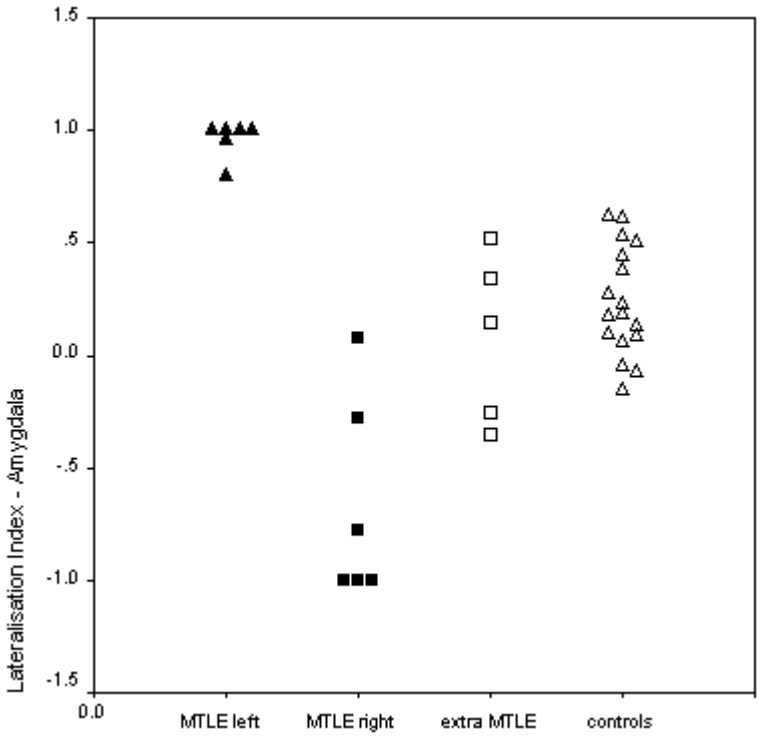

Figure 18: Plot of lateralisation indices of patients and controls. Note lateralised amygdala activation contralateral to the side of seizure onset in MTLE patients. In contrast, patients with epilepsy other than MTLE and healthy volunteers revealed lateralisation indices around zero.

The lateralisation indices of the amygdala were compared with activation induced by the Roland's Hometown Walking Task in the parahippocampal gyrus. In nine out of twelve patients, asymmetries of amygdala activation and parahippocampal activation were in accordance with asymmetries of parahippocampal activation (Table 3). Figure 19 exemplifies typical activation patterns in one patient with left- and one patient with right-sided MTLE.

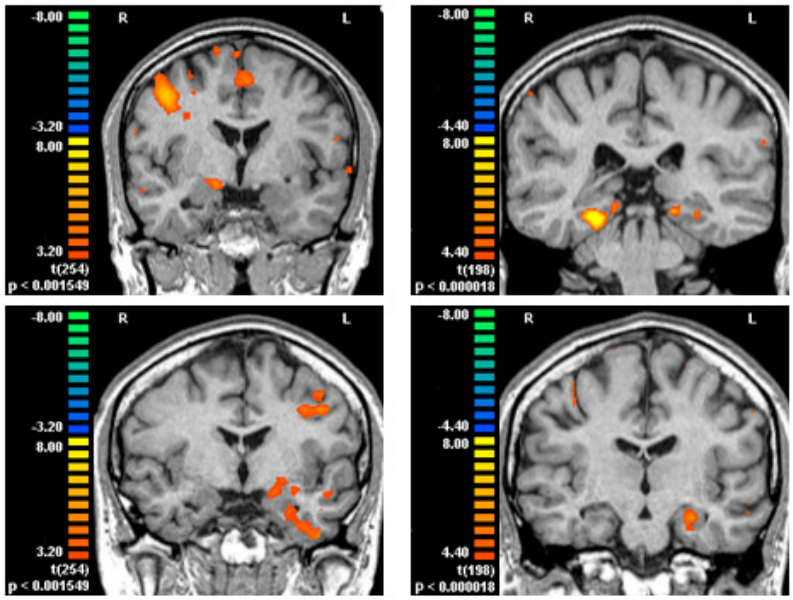

Figure 19: Example of a typical activation pattern within amygdala (left) and parahippocampal gyri (right) in left (above) and right-sided MTLE (below). Above: asymmetric activations contralateral to the seizure onset in a 33-year old woman with left-sided hippocampal sclerosis (left-sided language dominance, seizure onset at 13 years). Below: asymmetric activations contralateral to the side of seizure onset in a 25-year old man with right-sided hippocampal sclerosis (left-sided language dominance, seizure onset at 0 years).

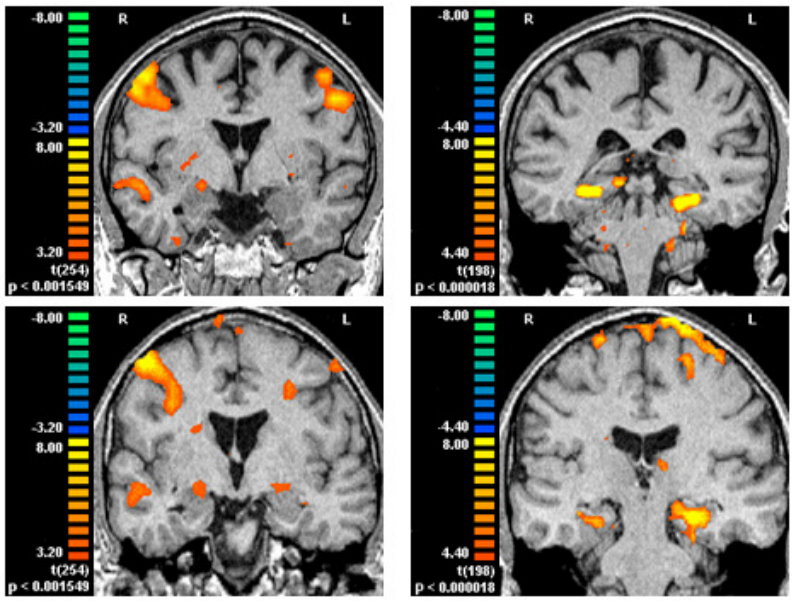

Figure 20: Example of dissociated activation pattern in amygdala (left) and parahippocampal gyri (right) in left-sided MTLE (above) and right-sided MTLE (below): Above: asymmetric amygdala activation contralateral to side of seizure-onset and reversed parahippocampal activation in a 45-year old man with left-sided MTLE (left-sided language dominance, seizure onset at 37 years). Below: amygdala was bilaterally activated while parahippocampal activation was asymmetric corresponding to the side of MTLE in a 41-year old man with right-sided hippocampal sclerosis (left-sided language dominance, seizure onset at 4 years).

In three patients we found dissociated amygdalar and parahippocampal activations. Dissociations were found in two directions: with unilateral amygdala and reversed asymmetries in parahippocampal activations (patient B2 with left (figure 20, above) and patient A6 with right-sided epilepsy) and bilateral amygdala-activation and asymmetric parahippocampal activation (patient A1 with left-sided MTLE; figure 20, below).

Finally, we analysed whether combining both the fearful face task and Roland's Hometown Walking Task improved the reliability of lateralisation in MTLE. Both paradigms were plotted against each other with LI of the parahippocampal gyrus (induced by Roland's Hometown Walking Task) on the x-axis and LI of the amygdala induced by the fearful face task on the y-axis (figure 21). The combination of both tasks separated MTLE patients from epilepsy patients other than MTLE and healthy volunteers.

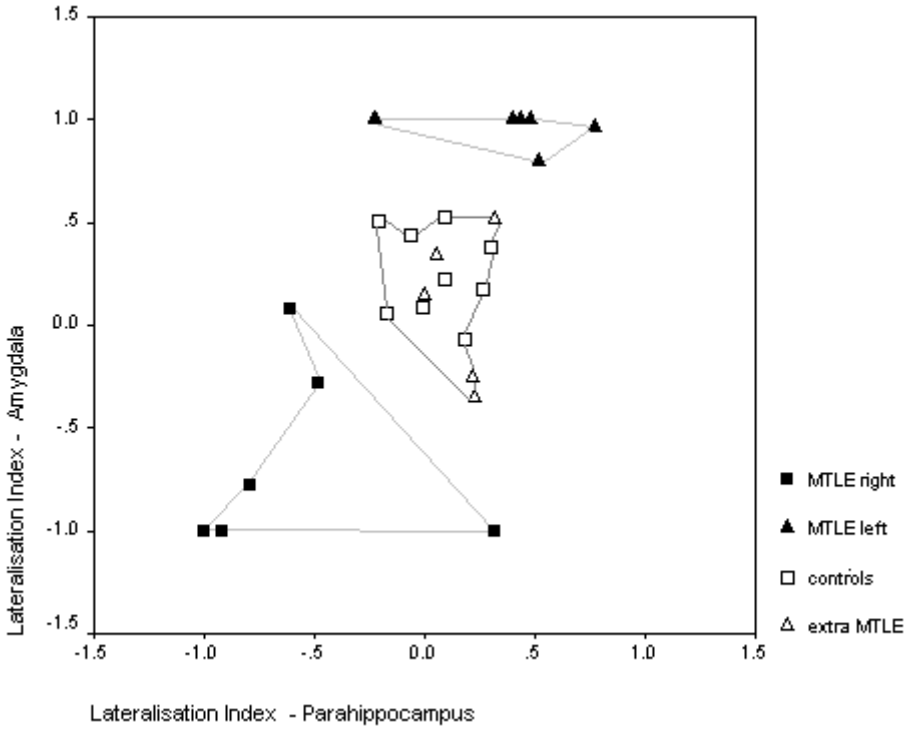

Figure 21: Lateralisation indices of the activations within parahippocampal gyrus (induced by Roland's Hometown Walking Task) and within amygdala (induced by the fearful face paradigm). The activation clusters of both paradigms separate patients with MTLE from healthy subjects and from patients with epilepsy other than MTLE

5.3.4 Discussion

This study investigated the feasibility, clinical usefulness and practicality of using fMRI imaging of the amygdala in individual patients with a fearful face paradigm. By using this paradigm in addition to Roland's Hometown Walking Task we hoped (1) to improve the reliability of MTL diagnostics in patients with refractory temporal lobe epilepsy and (2) to provide an imaging procedure that permits the evaluation of possible clinical implications for amygdala-related functions following temporal lobe surgery.

The feasibility of imaging the amygdala in individual cases was demonstrated by significant T2* contrast differences in the amygdala in response to watching sequences from thriller and horror movies showing actors portraying fearful faces in contrast to bland domestic landscape recordings. Amygdala activations were found in all subjects, including 17 epilepsy patients and 17 healthy volunteers. The activation focus was located in the upper portion of the amygdala, as has been reported in the literature (Breiter et al 1996; Pessoa et al 2002; Phillips et al 1997; Whalen et al 1998; Williams et al 2001). It should be mentioned that the procedure presented here was not aimed at identifying certain brain regions purported to be responsible for amygdala-activation by a single cognitive process. There is converging evidence, however, that motion (LaBar et al 2003), human faces in general and especially fearful facial expressions (Zald 2003) induce amygdalar activation in fMRI-experiments. Therefore, we conclude that the combination of these processes and the highly fitting context led to the considerable amygdala-activation we observed in our study.

An additional advantage of our paradigm for clinical applications is that it does not demand complex cognitive abilities, and that it can even be used in mentally impaired patients. The amygdala paradigm is easy to administer using commercially available equipment. Measurement of performance is not required, as the activation strength was not influenced by measured emotional involvement. This might be due to the fact that processing of fear-related sensory stimuli in the amygdala is highly automated (Dolan et al 2003). This has been reported in previous neuroimaging studies which found consistent activations in response to unattended fearful faces (Morris et al 2001).

The fearful face paradigm was analysed with regard to clinical features such as reliability, strength and reproducibility of activation in healthy volunteers. We found consistent and strong bilateral amygdala-activations in all 17 healthy volunteers. Cluster sizes were large enough to be detected in each individual. The reproducibility of amygdala activation was investigated by measurement-repetition in 12 healthy subjects. The activation extent and

strength was not reduced by repetition, although subjects remembered having seen the same scenes before. This suggests that there was no between-session habituation either in the number of significantly activated voxels or percent BOLD signal change. This finding apparently contradicts results of earlier studies showing that amygdala responses rapidly habituate (Zald 2003). This pattern of habituation has also been observed in fMRI experiments (Breiter et al 1996; Phillips et al 2001; Thomas et al 2001; Wright et al 2001). However, in contrast to our experiment, these studies used experimental designs with a single scan and static stimulus material. Furthermore, we found stable activation patterns in signal intensity and spread, indicated by high intraclass correlation coefficients. Retest-reliability of activation location was measured by the relative amount of overlapping activated voxels in two measurements, indicated by percentage overlap ($R^{ij}_{overlap}$). Overlap indices were only slightly lower compared to previous reported results of a language task (Fernandez et al 2003) and of an unattended primary visual task (Specht et al 2003). These findings could be due to the fact that the amygdala region is considerably smaller than the visual and language areas studied by these authors.

The fearful-face paradigm was then evaluated with regard to lateralisation-power. We found consistently strong and reliable bilateral amygdala activations in healthy volunteers and in patients with epilepsy not originating within the MTL. In contrast, the majority of MTLE-patients revealed clear interhemispheric asymmetries in amygdala activations, indicated by absent or very small clusters on the pathological side. It should be noted that the absence of activations was independent of the statistical threshold chosen, that is, no significant activations were found in activation maps with lower thresholds in those patients. From a methodological point of view it should be mentioned that individually adjusted thresholds would be appropriate because of the highly variable activation levels found in subjects and would, therefore, improve interpretability for patients with asymmetric activations. Patient A4, for example, elicited a high activation level and therefore had clearly asymmetric LI at higher thresholds (LI = -.74 on $p < .00001$), whereas the predefined threshold revealed weak asymmetry (LI = -.28 on $p < .001$). However, we preferred to present our data with predefined thresholds to allow for comparisons between subjects.

Analyses of amygdala-LI and parahippocampus-LI yielded comparable results. Lateralisation indices of amygdala-activation in fourteen out of seventeen patients was in accordance with memory-induced MTL-activations. Hemispheric asymmetries in the studied sample were more distinctive in the amygdala than in the parahippocampal gyrus. This could reflect possible dissociations which have been shown in structural studies.

Amygdala and hippocampal sclerosis are usually combined, but the sclerosis may also be solely restricted to the hippocampus or amygdala. This is one possible explanation for the findings in our first patients with a functional dissociation, in which the parahippocampus was active bilaterally while the amygdala was active unilaterally. On the other hand, the amygdala may escape the sclerotic process and be activated bilaterally, whereas activation of the parahippocampal region is clearly lateralised as shown in our second case of a dissociation. Further comparative studies of pathology of postoperative specimens might give us an explanation for the observed dissociation. This double dissociation of parahippocampal and amygdalar activation indicates that the paradigms used provide independent and supplementary lateralisation-information. This specificity is an important feature if the paradigm is to be used in a clinical context.

The combination of the fearful face task and the Roland's Hometown Walking Task, finally, highly increased the reliability of lateralisation in MTLE-patients. The increased reliability of functional MTL diagnostics may help to replace invasive IAT procedures by fMRI (Janszky et al 2005; Richardson et al 2004).

To summarise, the fearful face task presented here seems to be highly suitable for studying the amygdala in individual cases due to its reliability, reproducibility, specificity and lateralisation-power. The additional use of Roland's Hometown Walking Task improves presurgical diagnostics by increasing the reliability of lateralisation in patients with refractory MTLE. In addition, imaging the amygdala in a clinical context permits the evaluation of possible implications that temporal lobe surgery could have on various emotional and social variables.

6 General discussion and conclusion

The present report was aimed at investigating clinical fMRI applications within the mesial temporal lobe (MTL). These applications were designed to improve the clinical fMRI diagnostics in MTL epilepsy (MTLE). Comprehensive MTLE diagnostic includes the evaluation and prevention of negative treatment effects on patients' cognitive profiles. This is provided by neuropsychological examination and, increasingly, by fMRI. Mesial temporal lobe epilepsy is mainly treated with antiepileptic drugs (AED) or, when the seizures are refractory, by resective surgery. Both therapies may have substantial influence on cognition. The importance of evaluating cognitive side-effects of treatments is based upon the experience that impaired cognitive functions are one of the major influences on quality of life in individuals with epilepsy (Trimble 1994).

The studies reported here addressed three selected topics in the field of clinical fMRI within MTL. The first study approached a more technical question of fMRI. One of the major difficulties in using fMRI in a clinical context is the low BOLD contrast in individual subjects, and especially within MTL, where the SNR is genuinely low. Because caffeine has recently been proposed as an effective BOLD contrast booster in functional MRI studies for studies confronted with a low SNR (Mulderink et al 2002), we attempted to evaluate caffeine as a contrast booster for single-case fMRI-investigations.

The second study was aimed at improving the evaluation of pharmacological treatment of epilepsy. Antiepileptic drugs may have substantial cognitive side-effects. To date, neuropsychological examination has been the major method for objectively examining cognitive functions related to the use of AEDs. Functional MRI may improve diagnostics of pharmacologically induced cognitive side-effects. Before this can be realised, the fMRI-method needs to be validated with respect to the long-term reproducibility of the results. The second study was therefore aimed at investigating the intra-individual variability of serial fMRI-scans.

Probably the most important and the most advanced clinical application of functional MRI is found in the presurgical diagnostics of medically refractory MTLE patients. Functional MRI has been used to examine regional changes in brain function associated with seizures. The approach of functionally investigating the brain is based upon the fact that epilepsy is in many instances a functional disorder that must not necessarily be accompanied by gross abnormalities in structural imaging. In presurgical evaluation, fMRI is used mainly to assess localisation and lateralisation of language and memory to prevent postsurgical impairments. The third study was aimed at providing an fMRI

application to investigate additionally the amygdala. This could improve the reliability of MTL diagnostics in MTLE patients and may in future allow for the evaluation of possible clinical implications of amygdala-resections, such as emotional and social impairment following temporal lobe surgery.

6.1 Evaluation of the aims

6.1.1 First study

The first study, investigating whether caffeine might be used as a BOLD contrast booster in clinical studies, uses a single case, placebo controlled, repeated fMRI study design. Six healthy volunteers performed eight fMRI sessions on four different days. Each study day consisted of a pretreatment session prior to the application of placebo or caffeine and a post-treatment session. Each fMRI session included a visual, a motor and a memory task.

The first aim of this study was to replicate previously reported group results, where caffeine obviously boosted the BOLD contrast within the primary motor and the visual area (Mulderink et al 2002). We hypothesised that the application of caffeine is efficient to boost the BOLD contrast on a single case level. The study confirmed through group analyses that 200mg caffeine enhanced the BOLD-contrast in M1 during the performance of a motor task (ROI-GLM: $p < .001$), however the more conservative repeated measures ANOVA failed to confirm this result ($p = .09$). Caffeine increased the inter-individual variability of percent BOLD signal change compared to the baseline condition. We failed to reproduce the reported signal increase following administration of caffeine in V1. Instead, group analyses showed that caffeine decreased the BOLD-contrast in V1 ($p < .00001$). At an individual level we found increases as well as decreases in the BOLD-signal in V1. This was statistically confirmed by applying a RMANOVA model ($p = .81$).

The second aim of the study was to investigate whether caffeine does enhance the BOLD contrast within MTL and we explored whether enhancing effects will be observable on a single-case level. We found within the MTL no significant change following caffeine intake, neither for group analyses ($p = .65$) nor in the RMANOVA ($p = .20$).

It should be mentioned that a higher dose of caffeine would be more effective in influencing the BOLD signal. The 200mg dose was in our study chosen in accordance to Mulderink and co-workers (2002) and Laurienti and co-workers (2002) who administered a caffeine dose in this range, namely 200mg (Mulderink et al 2002) and 250mg (Laurienti et al 2002). However, a higher dose is not practical in a clinical setting due to adverse side-

effects. It has been reported that subjects felt jittery, anxious or nervous after the administration of doses exceeding 300mg (Evans et al 1991).

One should take into account that our results are based on a small sample. However, it was our intention to investigate intra-individual effects of caffeine administration. The use of caffeine as a BOLD contrast booster in a clinical context requires reliable successive results in each individual subject. Previous studies referred to group designs without administration of both placebo and caffeine conditions (Mulderink et al 2002) or pre- and posttreatment caffeine conditions within a single subject (Laurienti et al 2002). In contrast, we chose an extended study design with each person acting as their own control in four baseline, two placebo and two caffeine measurements.

To summarise, caffeine seems to be far less effective as a BOLD contrast booster than expected, although previous studies found boosting effects on the BOLD response following caffeine administration. Our results discourage the use of caffeine in a clinical context as it may enhance inter-individual variability and may even reduce the signal in certain subjects. Moreover, in our study caffeine related BOLD-contrast changes were clearly domain specific within individual subjects.

6.1.2 Second study

The second study investigated the longterm-reproducibility of serial fMRI scans within the MTL. Six healthy volunteers were examined six times with a modified version of the Roland's Hometown Walking Task (Jokeit et al 2001c). The scans were performed on four different days with intervals of 1-4 weeks.

First, we aimed to describe the magnitude of variability of MTL activations over serial scans both in single-subjects and in group analyses. On single-subject level, we found a high variability, indicated by high variance coefficients in the significantly activated voxels and the percent BOLD signal change. None of the studied subjects revealed stable results over all repeated scans. Our study clearly demonstrated that quantitative interpretation is not a reliable method of studying longterm effects of brain function at single-subject level. In contrast, the results on the group level were more promising. We found reasonable reproducibility and sufficient intraclass correlation coefficients. Nevertheless, the observed amount of variance was considerable and could not be explained by several experimentally (e.g. diurnal factors, cycle phase, nutrition habits, drug intake) or statistically controlled variables (e.g. repetition or behavioural effects). Knowledge about the magnitude of variability can help in future AED studies to distinguish between

variations inherent in the examination method and changes in the subject's brain induced by pharmacological treatment.

Second, we explored whether the magnitude of the observed variability would allow future investigations of treatment effects of AEDs on the MTL. The considerable amount of variability in fMRI-data over a series of scans implies that meaningful data can only be expected when strong treatment effects are present. The averaged relative deviations between scans in our study was between 30% and 40%. Two recent pharmacological fMRI studies probably used potent substances and therefore observed substantial variations in the BOLD response (Jokeit et al 2001b; Sperling et al 2002). Both of the anticholinergic drugs lorazepam and scopolamine (Sperling et al 2002) and the antiepileptic drug carbamazepine (Jokeit et al 2001c) decreased the BOLD signal within the MTL. Compared to a placebo-condition, lorazepam and scopolamine decreased the significantly activated voxels within the hippocampus by approximately 40-60% and the percent BOLD signal change by approximately 50-60%. When only high doses of carbamazepine (serum levels of more than 7 mg/l) are included, the proportion of significantly activated voxels within the MTL was decreased by approximately 50% as compared to a control group. Using serial scans and group analysis therefore appears to be a realistic method for investigating cognitive side-effects of AEDs on the MTL.

To summarise, we observed highly variable fMRI results on single-case level. The quantitative interpretation is therefore not a reliable method to individually diagnose AED changes on brain functions. However, at a group level, we found sufficient reproducibility. The results do not discourage the application of serial investigations to study the influence of long-lasting AED treatments. But to date, the application is limited to its use in longitudinal group studies and to the evaluation of strong influences on the brain functions, such as could be expected with high doses of AEDs.

6.1.3 Third study

The third study investigated the feasibility and usefulness of imaging the amygdala. We developed a fearful face fMRI-paradigm that included the visual presentation of sequences from thriller and horror movies showing actors portraying fearful faces. First, we aimed at evaluating the feasibility of imaging the amygdala in single subjects and investigated 17 patients with symptomatic focal epilepsy (12 had MTLE (6 right- and 6 left-sided)) and in 17 healthy control subjects. The fearful-face paradigm led to significant activations ($p < .001$) of the amygdala in all subjects, including 17 patients with sympto-

matic focal epilepsy (12 had MTLE (6 right- and 6 left-sided)) and 17 healthy control subjects.

Second, it was aimed at investigating whether the paradigm used fulfils prerequisites of clinical diagnostics. The results clearly demonstrated, that the paradigm fulfilled pre-requisites of strong and reliable activations observed in all studied subjects. Additionally, reproducibility of amygdala activation was demonstrated by restudying 12 of the control subjects one to eight weeks later. We found stable activation patterns indicated by high interclass correlation coefficients and considerable retest-reliability of activation location, measured by the relative amount of overlapping activated voxels in two measurements. The easy administration and the low cognitive demand on patients increases the practicality of this tool for studying amygdala-functions and deficits in a clinical context. Even more, measurement of performance is not required, as far as in our study the activation strength was not influenced by measured emotional involvement. This might be due to the fact that processing of fear-related sensory stimuli in the amygdala is highly automated (Dolan et al 2003).

Third, it was attempted to investigate whether two distinctive MTL paradigms improve the reliability of MTL diagnostics. We therefore additionally measured parahippocampal activations using the Roland's Home Town Walking Task (Jokeit et al 2001c) within the same session in all 17 patients and in nine of the control subjects. Amygdala activations were clearly lateralised in MTLE patients and bilateral in control subjects, including patients with epilepsy other than MTLE, and healthy volunteers. Comparing the results of the fearful face paradigm with the results obtained by the Roland's Hometown Walking Task, we found double dissociated activations of amygdalae and parahippocampi in three MTLE patients, indicating that these tests provide independent and specific information. Finally, combining both paradigms highly improved the reliable lateralisation of the epileptogenic focus in MTLE.

To summarise, the aims of the third study were clearly fulfilled. The amygdala activation brought on by our fearful face paradigm was strong, robust, reproducible, and specific in individual subjects. The feasibility of imaging the amygdala in individual cases allows one to evaluate possible clinical outcomes of amygdala-related functions such as emotional and social functioning following TL surgery. The combination of the fearful face paradigm and the Roland's Hometown Walking Task increases the reliability of lateralisation in MTLE patients and thus provides a more detailed and reliable presurgical mapping of MTL structures.

6.2 General discussion and outlook

The highest benefit for clinical fMRI diagnostics from the present thesis is certainly the demonstrated feasibility of imaging the amygdala in a clinical context. The highly increased reliability of lateralisation by the use of two distinctive paradigms, the fearful face paradigm and the Roland's Hometown Walking Task, is extremely useful in the field of presurgical diagnostics of refractory MTLE-patients. The increased lateralisation-power may help to decrease the number of invasive and risky IAT procedures. To date, fMRI of memory-lateralisation is far from replacing the invasive IAT. With recent procedures, the reliability of lateralisation was no higher than about 90% (Killgore et al 1999). Therefore, our results of increased lateralisation-power are promising. Certainly this method demands further external validation regarding the predictive value of fMRI for postsurgical outcome. It still needs to be demonstrated that the fMRI procedure of applying both paradigms reveals typical and atypical results in large numbers of individual cases in agreement with a standard, i.e. the IAT in presurgical diagnostics or the postoperative outcome. When prospective studies are clearly able to demonstrate that resection in a region of fMRI activation results in a deterioration in memory performance, fMRI data could be used in planning specific resections in individual cases. Accordingly, recent preliminary studies have demonstrated the feasibility of assessing the fMRI memory activation in predicting postsurgical memory performance in TL patients (Janszky et al 2005; Richardson et al 2004). Additionally, fMRI results from ten left-sided MTLE patients provided a stronger predictor of memory outcome after surgery as compared to other measures, i.e. the hippocampal volume and pre-operative verbal memory function.

The greatest impact of the amygdala-study is the possibility to evaluate the implications that temporal lobe surgery could have on various emotional and social variables. At first glance, epilepsy surgery appears to cause no clinically salient alterations such as deterioration in psychosocial status. However, the number of controlled studies using standardised measures to examine social and emotional outcome associated with epilepsy surgery are few (Hermann 2002) and the emphasis of most studies is on overall group comparisons. Therefore, a closer examination of surgical patients may reveal individuals who show no improvement and may even deteriorate in terms of psychosocial status (Hermann 2002). In children and young adults, however, low occupational or educational status (Keene et al 1998) and long-term problems in social functioning (Jalava et al 1997; Williams et al 1998) have been reported, even when seizures are eliminated or significantly reduced. However, problems in emotional processing, including social behaviour and cognition following unilateral TL surgery, seem to be more subtle and

psychometrically difficult to detect and are probably limited to specialised functions. Recent studies in epilepsy patients have suggested impaired abilities in social perceptual skills such as recognition of negative emotions from facial expressions (Adolphs et al 2001; Anderson et al 2000). Others have found no deterioration in theory of mind-tests, including reasoning about the mental states of others, in subjects who sustained surgical damage to a previously normal amygdala in adult life (Shaw et al 2004). In contrast to the controversial results following unilateral amygdala-damage, bilateral damage has received more attention in the literature. It is associated with impairments in the conditioning of autonomic responses (Bechara et al 1995), in emotional memory (Brierley 2004; Cahill et al 1995), and in social perceptual skills such as recognising facial expression (Adolphs 2003) and emotional behaviour (Tranel et al 1990). Similar to patients with acquired bilateral amygdala damage, epilepsy patients with dysfunction of the remaining amygdala could be at risk for impairments in their emotional processing. However, prospective outcome studies are needed to assess whether the amygdala is involved in patients who show deteriorations in emotional processing. Imaging the amygdala presurgically may help to tailor epilepsy surgery in patients with refractory MTLE. Clearly this approach would require prospective validation, but patients undergoing presurgical evaluation represent an ideal population to study whether fMRI activations are conclusively related to specific task function.

The benefit of the present thesis for the improvement of pharmacological treatment evaluation might be less obvious. In contrast to presurgical diagnostics, the current state of research regarding the use of fMRI in the evaluation of pharmacological treatments is far less sophisticated. The use of fMRI in neuropharmacological studies is in its very beginning, especially for long-acting drugs. In addition, there is little research into the quantitative reproducibility of serial fMRI results, a substantial prerequisite for such studies. This is certainly due to the fact that such studies are very time-consuming, costly and laborious. Additionally, they could turn out to be difficult, because of genuinely high variability in the fMRI-data due to factors which are not fully controllable (i.e. scanner stability or physiological influences). Despite this unfavourable situation, the use of fMRI for examining cognitive side-effects needs to be investigated because of the need for improved clinical diagnostics and because fMRI allows one to diagnose cognitive side effects more objectively than traditional neuropsychological examination.

Because of these limitations, the study reported in this thesis had to be directed at more basic and technical issues, i.e. the longterm-reproducibility of serial fMRI scans within the MTL both in single-case and in group analysis.

On single-subject level, the intra-individual variability of fMRI-results within MTL was high and corroborated previous findings from primary sensory and motor areas (McGonigle et al 2000; Waldvogel et al 2000). To date, the technical capabilities of fMRI do not allow for diagnosis of cognitive AED side-effects on an individual basis. One probable solution would be to interpret the data not quantitatively within one structure but qualitatively, in terms of the activation pattern. We did not investigate the activation pattern induced by the Roland's Hometown Walking Task in areas other than the MTL. There is one study investigating the qualitative reproducibility of memory-related activation pattern (Miller et al 2002). The authors observed highly variable activation patterns from individual to individual, but despite these large variations the intra-individual activation patterns were reliable over time. The authors suggested that individuals may have been using very different strategies and cognitive processes during the task and those differences were reflected by different patterns of brain activations. On-off patterns of the MTL activations might be interesting for studying severe influences on memory-related MTL structures, where compensation strategies are needed.

By analysing group-results, we found sufficient reproducibility over several scans. Although the results of group studies are not necessarily representative for each individual subject, they might improve the diagnostics of pharmacologically induced cognitive disturbances by diminishing the impact of any confounding variables. For example, the adverse effects of AEDs on memory may be offset by reductions in seizures and interictal discharges, because treating the seizures with AEDs may have positive effects on memory performance. In addition, the effects of AEDs may interact with focal brain dysfunctions (Durwen et al 1993). Another factor affecting the results is mood, which may alter the objective memory performance in epilepsy patients (Motamedi et al 2004). Functional MRI would allow one to directly investigate the functionality of memory-related MTL structure and to avoid the problem of overt verbal or non-verbal responses that might be modulated by individual states and traits.

The benefit for pharmacological treatment evaluation is indirect but could aid future studies of pharmacological treatment effects of AEDs. The study allows one to distinguish treatment effects from variations inherent in the examination method and is useful for the planning of study designs. Based on our findings, we recommend the use of longitudinal group analyses, using serial scans. Longitudinal studies that cover initiation, chronic administration and withdrawal of AEDs are methodologically robust designs.

Hereafter, it would be most evidently to prove the effects of carbamazepine within the MTL by using fMRI. A number of studies have shown that high doses have a substantial

effect on memory and the related MTL structures. Two independent studies, using SPECT and Doppler sonography have demonstrated decreased cerebral blood flow changes due to carbamazepine (Futagi et al 1994; Sechi et al 1995). This might also be associated with reduced cognitive performance. By comparing the variances found in our reproducibility study with the carbamazepine-effects previously observed in a cross-sectional fMRI study, we have shown that fMRI would allow one to study cognitive side-effects of AEDs on MTL-structures.

The benefit for clinical fMRI diagnostics from the caffeine-study, namely boosting the BOLD signal within the MTL, was far below our expectations. We failed to find any effects within this area following caffeine administration. Unfortunately, it is precisely the MTL where a boosting effect from caffeine would be of greatest help for clinical diagnostics. In contrast to primary sensory and motor regions, where significant percent BOLD signal changes can be easily and robustly invoked, the BOLD signal within the MTL is by nature small and difficult to obtain in single subjects (Powell et al 2004). Our results, however, clearly failed to show any consistent boosting effect of caffeine within the MTL.

However, the activation magnitude induced by the Roland's Hometown Walking Task does not necessarily need to be boosted because this paradigm commonly induces reliable and strong activations within MTL, even when conservative thresholds are used. The disadvantage of this paradigm, and for block-designs in general, is that it is not possible to extract brain activations related to correct answers. Event-related paradigms in contrast, where this goal is achieved, reveal signal changes that are too weak within single-case fMRI in MTL. The use of caffeine would have been a very easy and safe method of boosting the signal and improving clinical diagnostics at 1.5T. However, our results discount the use of caffeine as a BOLD contrast booster in a clinical context. Therefore, other approaches such as technical improvements are required to increase the fMRI-sensitivity for clinical diagnostics. The most realistic approach is probably the application of higher field strength magnets such as 3T, assuming that its application becomes practicable for clinical fMRI diagnostics. To date, only one study is available that demonstrates the possibility of imaging epilepsy patients at 3T (Szaflarski et al 2004). As the study had a small sample size (no more than six patients), the data are preliminary and the utility of high field fMRI in a clinical context needs to be investigated in a larger population.

6.3 Conclusion

The studies presented here evaluated fMRI paradigms that result in improvements in clinical diagnostics of MTLE. We were able to considerably improve the presurgical diagnostics in MTLE patients by demonstrating the feasibility of imaging the amygdala with fMRI in individual subjects. By employing dynamic fearful faces contrasted by bland landscapes our paradigm activated both amygdalae strongly, robustly, replicably and specifically in individual subjects. The ease of administration and the low cognitive demand on patients increases the practicality of this tool for studying amygdala-functions and deficits in a clinical context. The feasibility of imaging the amygdala in individual cases allows for the evaluation of possible clinical implications for amygdala-related functions such as emotional and social processing following TL surgery. The combination of the fearful face paradigm and the Roland's Hometown Walking Task increased the reliability of lateralisation in MTLE patients and, therefore, provides a more detailed and reliable presurgical mapping of MTL structures.

Regarding the diagnostics of pharmacologically induced cognitive dysfunctions, we were able to plan future pharmaco-fMRI studies targeting cognitive side-effects of AEDs. Using six serial fMRI-scans over several weeks, the present study specified the variability inherent in the long-term-stability of fMRI results within the MTL. Despite considerable variability over the scans we suggest that the investigation of long-lasting AED treatments on the MTL is feasible and reasonable with the use of long-term group studies. Future studies could improve the understanding of mechanisms, which result in pharmacologically induced cognitive disturbances.

The aim of improving the genuinely low BOLD signal within MTL through the use of caffeine clearly failed. Therefore, other approaches such as technical improvements are required to increases the fMRI- sensitivity for clinical diagnostics. The most realistic application is probably the use of higher field strength magnets such as 3T, assumed that its application becomes practicable for clinical fMRI diagnostics.

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8 Publications

8.1 Papers

Schacher, M., H. Jokeit (2004). Neuropsychologie in der Epileptologie. Aktuelle Neurologie 31(2), 73-78.

Jokeit H., M. **Schacher** (2004). Neuropsychological aspects of type of epilepsy and etiological factors in adults. Epilepsy and Behavior, 5 (Suppl 1), S14-20.

Schacher, M., Huber, D., H. Jokeit (2004). Funktionelle Bildgebung in der klinischen Neuropsychologie. Zeitschrift für Epileptologie, 21, 82-88.

Schacher, M., Hämmerle, B., Woermann, F. G., Okujava, M., Huber, D., Grunwald, T., Krämer, G., H. Jokeit (in press). Amygdala fMRI lateralises temporal lobe epilepsy. Neurology.

Schacher, M., Okujava, M., Zaugg, P., Krämer, G., H. Jokeit (submitted). Coffee cantata: On the use of caffeine as a contrast booster for BOLD fMRI studies.

8.2 Posters at scientific meetings

Schacher, M., Regard, M. (2001). Anatomo-functional correlates of 90 degree rotation drawing errors in brain-damaged patients. Poster at ZNZ Symposium, Zurich.

Schacher, M., Sälke-Kellermann, R.A., Lang, P., Harms, D.O., H. Jokeit (2003). Profile of cognitive improvement after VPA withdrawal. A case-report. Poster presented at: Gemeinsame Jahrestagung der Internationalesn Liga gegen Epilepsie, Berlin.

Schacher, M., Kötz, C., Osterkamp, G., Krämer G., Huber D., H. Jokeit (2003). Längerfristige Stabilität der BOLD-Antwort in mesialen Temporallappenstrukturen-Interindividuelle Vergleiche. Poster presented at: Jahrestagung der deutschen Gesellschaft für klinische Neurophysiologie, Freiburg/Breisgau.

Jokeit H., **Schacher** M., Hämmerle B., Kötz C., Osterkamp G., Porcellini B., D. Huber (2003). Amygdala-Aktivierung durch Videosequenzen mit Gesichtern furchterfüllter Menschen. Poster presented at: Jahrestagung der deutschen Gesellschaft für klinische Neurophysiologie, Freiburg/Breisgau.

Schacher, M., Kötz, C., Osterkamp, G., Hofmann, C., Krämer G., Huber D., H. Jokeit (2003). Longterm-stability of BOLD-response in the mesial temporal lobe. Poster

presented at: The 6th European congress on Epileptology of the International League against Epilepsy, Vienna.

Jokeit, H., **Schacher** M., Hämmerle B., Kötz C., Osterkamp G., Hofmann, C., Porcellini B., D. Huber (2003). Video sequences of fearful faces reliably activate amygdalae in single subjects. Poster presented at: The 6th European congress on Epileptology of the International League against Epilepsy, Vienna.

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9 Curriculum vitae

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Ausbildung:

1988 bis 1995	Kantonsschule Alpenquai, Matura Typus B
1995 bis 1997	Psychologie Grundstudium, Universität Fribourg
1997 bis 1998	Praktikumjahr
1998 bis 2001	Hauptstudium Psychologie und Lizentiat, Universität Fribourg Fächer: Klinische Psychologie, Allgemeine Psychologie, Neurobiologie
2002 bis 2005	Dissertation zum Thema „Evaluation of clinical fMRI paradigms within the mesial temporal lobe“ am Schweizerischen Epilepsiezentrum unter der Leitung von PD Dr. H. Jokeit und Prof. Dr. L. Jäncke
2002 bis 2005	PhD Studium am Zentrum für Neurowissenschaften Zürich

Praktika / Berufliche Tätigkeit:

1997 - 1998 Praktika:

- Berufs- und Weiterbildungsberatung des Kt. Luzern (1 Monat)
- Schweizerisches Paraplegikerzentrum Nottwil, Beratungsdienste (3 Monate)
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